IMPROVING NITROGEN EFFICIENCY THROUGH ENHANCED UREA-NITROGEN RECYCLING IN RUMINANTS

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ABSTRACT

Three experiments were conducted to study dietary effects on urea-nitrogen (N) recycling as a strategy to improve the efficiency of N utilization in ruminants. Experiment 1 examined the effects of feeding diets containing two levels of dietary crude protein (CP; 10.8 vs. 14.0%) and ruminally-degradable protein (RDP; 73.4 vs. 76.0% of CP) on urea-N recycling to the gastro-intestinal tract (GIT), N balance, and microbial protein production in beef heifers. Feeding the low CP diet decreased N intake ($P < 0.01$), ruminal ammonia-N (NH_3-N) concentration ($P < 0.01$) and urinary N excretion ($P < 0.01$). Endogenous urea-N production increased ($P = 0.03$) with increasing dietary CP level, as did urinary urea-N loss ($P = 0.04$). However, urea-N transfer to the gastro-intestinal tract (GIT) was similar across diets, with most of this N returned to the ornithine cycle. Microbial N supply was unaffected ($P > 0.05$) by dietary treatment. Experiment 2 examined the effects of feeding diets containing two levels of ruminally-degradable starch (RDS; 28.6 vs. 69.2% of total starch) and RDP (48.0% vs. 55.0% of CP) on urea-N recycling to the GIT, N balance, duodenal nutrient flow, and ruminal microbial protein production in beef heifers fed low CP (10%) diets. Ruminal NH_3-N concentration was greater ($P = 0.01$) in heifers fed high RDP as compared with those fed low RDP, and it was also greater ($P = 0.01$) in heifers fed low RDS as compared with those fed high RDS. Microbial N flow to the duodenum increased as RDP level increased on the high RDS diet, but was not affected by RDP level on the low RDS diet (interaction; $P = 0.04$). Urea-N entry rate and urea-N transfer to the gastro-intestinal tract were similar ($P > 0.05$) across diets. The amount of recycled urea-N that was incorporated into microbial N increased as RDP level increased on the high RDS diet, but the opposite was observed on the low RDS diet (interaction; $P = 0.008$). Experiment 3 examined the effects of feeding diets containing two levels of CP (14.9 vs. 17.5%) and RDP (63.0 vs. 69.0% of

CP) on urea-N recycling to the GIT, microbial protein production, N balance, omasal nutrient flow, and milk production in lactating dairy cows. Nitrogen intake ($P < 0.01$) and both urinary N ($P < 0.01$) and urea-N ($P < 0.01$) output were greater for cows fed the high compared with those fed the low CP diet. Ruminal NH_3-N concentration tended to be greater in cows fed the high than those fed the low CP diet ($P = 0.06$), and was greater in cows fed high RDP as compared with those fed the low RDP diet ($P < 0.01$). However, N balance, milk yield, and microbial N supply were unaffected ($P > 0.05$) by dietary treatment. The proportion of endogenous urea-N that was recycled to the GIT (i.e., GER: UER) was greater ($P = 0.02$) in cows fed the low CP compared with those fed the high CP diet. In summary, the results of this thesis show that reducing dietary CP level in beef and dairy cattle reduces urinary N excretion whilst maintaining microbial N supply. In addition, judicious combinations of RDP and RDS when feeding low CP diets can potentially enhance the efficiency of microbial N production. These data show that through careful dietary manipulation, overall efficiency of N utilization can be improved leading to a reduction in N excretion into the environment.

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LIST OF ABBREVIATIONS

[$^{15}N^{15}N$]-urea	Double-labelled urea
AA	Amino acids
ADF	Acid detergent fiber
ADIA	Acid detergent insoluble ash
ADIN	Acid detergent insoluble nitrogen
AOAC	Association of Official Analytical Chemists
BW	Body weight
CP	Crude protein
DM	Dry matter
FP	Fluid phase
GER	GIT entry rate (amount of recycled urea-N entering the GIT)
GIT	Gastro-intestinal tract
ILO	Intensive Livestock Operation
LP	Large particle phase
MUN	Milk urea nitrogen
N	Nitrogen
NA	Natural abundance
NAN	Non-ammonia-nitrogen
NDF	Neutral detergent fiber
NDIN	Neutral detergent insoluble nitrogen
NH_3	Ammonia
NH_4^+	Ammonium ion

NO_2	Nitrogen dioxide
N_2O	Nitrous oxide
NPN	Non-protein nitrogen
OM	Organic matter
OMTDR	Organic matter truly digested in the rumen
OTD	Omasal true digesta
PD	Purine derivatives
PUN	Plasma urea-nitrogen
RDP	Ruminally-degradable protein
RDN	Ruminally-degradable nitrogen
RDS	Ruminally-degradable starch
RFC	Ruminally-fermentable carbohydrate
ROC	Urea-nitrogen reentering the ornithine cycle in the liver
RUP	Ruminally-undegradable protein
SP	Small particle phase
TMR	Total mixed ration
UER	Urea-N entry rate (total endogenous urea-N production)
UFE	Urea-N in faeces
UT	Urea transporter(s)
UUA	Urea-N utilized for anabolism
UUE	Urinary urea-N elimination
UUN	Urinary urea-N
VFA	Volatile Fatty Acids

1 GENERAL INTRODUCTION

Improving the efficiency of nitrogen (N) utilization in ruminant animals is an important factor in reducing feed costs and mitigating the negative environmental impact of intensive livestock operations (ILO). Ruminants are relatively inefficient at utilizing dietary N. For example, in beef cattle approximately 25% of dietary N is retained in tissue, with the remainder being excreted in the faeces (29%) and urine (39%) (Bierman 1999; Gaylean 1996). In dairy cows, 25 to 30% of dietary N is deposited in milk protein, with 70 to 75% excreted in the faeces and urine (Tamminga 1992). The excretion of excess dietary N, particularly as urinary N, can have a negative impact on the environment. It has been estimated that 50 to 90% of urinary N is in the form of urea-N (Reynal and Broderick 2005). Once excreted, urea-N is rapidly volatilized to ammonia, and can be converted to nitrous oxide (N_2O) which is an important greenhouse gas (Janzen et al. 2003; Baggs and Philippot 2010; Hristov et al. 2011). Urinary N can also leach into soil and ground water as nitrate (Tamminga 1992; Socolow 1999; Cowling and Galloway 2002). Faecal N is mostly present in a complexed organic form that is less volatile than urinary N, and therefore, partitioning more excreted N to faeces rather than urine offers an opportunity to reduce environmental pollution (de Klein and Ledgard 2005). Furthermore, reducing the amount of feed that is required to produce a unit of meat or milk reduces feed costs and improves overall production efficiency (Chandler 1996). Efficiency of nutrient utilization is becoming more important as demand for livestock products increases due to increased population growth and the increasing affluence of developing countries (Steinfeld et al. 2006; de Klein et al. 2010). As well, there is increasing public concern over sustainable agricultural production practices (Janzen 2011). Several strategies can be used to address the issue of inefficient N usage in ruminants, including reducing dietary crude protein (CP) concentration, increasing microbial capture of

recycled N, and feeding management practices such as feeding frequency and the feeding of a total mixed ration (TMR) (Tamminga 1992; Castillo et al. 2000; Lapiere and Lobley 2001; Flachowsky and Lebzien 2006; Hristov et al. 2011).

Extensive ruminal degradation is contributes significantly to the inefficient utilization of dietary protein. Dietary protein arriving in the rumen is degraded by ruminal microorganisms to peptides, amino acids (AA) and ammonia (NH_3) which can be used by some ruminal microbes to synthesize microbial protein (Bach et al. 2005). However, ruminal microorganisms degrade dietary protein regardless of microbial requirements for N, potentially resulting in excess ammonia-N (NH_3-N) in the rumen (Leng and Nolan 1984). Ammonia can become toxic if allowed to accumulate in the tissues and can even lead to death. Therefore, NH_3 in excess of microbial requirements is absorbed across the ruminal wall and transported to the liver via portal blood where it is detoxified by conversion to urea-N through the urea cycle and is subsequently transported to the kidneys for excretion in the urine or it can be recycled to the gastrointestinal tract (GIT) either through blood of saliva (Lapierre and Lobley 2001).

The recycling of urea-N to the GIT is an important salvage mechanism for ruminants because if all endogenously produced urea-N was excreted, it would represent a substantial and irreversible loss of N to the animal and could lead to a negative N balance or N deficiency (Stewart and Smith 2005). Urea-N recycled to the GIT can be hydrolyzed to NH_3 by microbial enzymes and then utilized as a source of N for microbial protein synthesis, which is a major source of AA for maintenance and productive functions in the host (Lapierre et al. 2006). Therefore, enhancing urea-N recycling to the GIT is a potential opportunity to improve the efficiency of N utilization in ruminants. There are several dietary and ruminal factors that affect the rate of urea-N recycling to the GIT and its incorporation into microbial protein. These

include the concentration of dietary protein and N intake (Bunting et al. 1987; Marini and Van Amburgh, 2003; Marini et al. 2004), dry matter (DM) intake (Sarraseca et al. 1998), forage-to-concentrate ratio (Huntington et al. 1996), and dietary levels of ruminally-degradable protein (RDP) (Wickersham et al. 2008a; Rémond et al. 2009), and ruminally-fermentable carbohydrate (RFC; Kennedy and Milligan 1980; Huntington 1989; Huntington et al. 2009). Ruminal factors such as NH_3-N concentration, bacterial urease activity, concentrations of volatile fatty acids (VFA), carbon dioxide (CO_2) concentration, urea transporters, and pH also effect the movement of blood urea-N into the rumen (Kennedy and Milligan, 1980; Abdoun et al. 2010). However, there is limited information on how simultaneous changes in these factors influence the recycling of urea to the GIT and its subsequent incorporation into microbial protein in both beef and dairy cattle. Therefore, the goal of this thesis was to investigate how manipulation of dietary CP, RDP and RFC levels can influence urea-N recycling in ruminants with the aim of improving overall N efficiency.

2 REVIEW OF LITERATURE

2.1 Introduction

Modern dairy and beef operators employ intensive management systems leading to the concentration of large numbers of animals in a confined area. The goals of such management systems are to improve production efficiency, but they can also lead to environmental concerns when cattle are not fed and managed properly (NRC 2001). One of the goals of ration formulation models is to provide cattle with the necessary level of nutrients to achieve a certain level of production (NRC 1996; NRC 2001; Fox et al. 1992). A nutrient deficiency could result in lowered production, whereas overfeeding can lead to the excretion of excess nutrients into the environment and increase the cost of production (Chandler 1996; Hristov and Jouany 2005). Therefore, maximizing nutrient utilization is important in terms of managing livestock operations. For example, when dietary protein is fed in excess of requirement, it is not used efficiently for milk or meat production and the excess is excreted into the environment via the faeces and urine, resulting in pollution and increased costs of production (Chandler 1996). Not only is excess N an environmental problem it also represents an irreversible loss of nutrients from the host (James et al. 1999). Faecal N is less volatile in the environment than urinary N therefore management strategies that reduce overall N excretion and partition more excess N to the faeces could potentially reduce the environmental impact of intensive livestock production systems (de Klein and Ledgard 2005). Optimizing microbial protein production through the enhancement of urea-N recycling in ruminants is one such strategy that requires further investigation.

2.2 Ruminal Degradation of Dietary Protein

The ruminant digestive tract provides an anaerobic environment suitable for the growth and proliferation of a complex variety of microorganisms including eubacteria, archaebacteria (methanogens), fungi and protozoa (Hobson and Stewart 1997). This highly diverse microbial population secretes a wide array of enzymes that enable them to digest plant material from a variety of sources and in turn provide the host animal with an energy source (i.e., VFA) and high quality protein (i.e. microbial protein), resulting in a mutually beneficial symbiotic relationship (McAllister et al. 1994; Wallace et al. 1997; Krause et al. 2003).

Figure 2.1 represents N metabolism in the rumen where feed protein is ingested and utilized by the ruminal microbial population or might escape degradation and pass to the lower intestinal tract. Feed nitrogen entering the rumen can be classified as either true protein or non-protein nitrogen (NPN). True protein consists of both RDP and ruminally-undegradable protein (RUP). Ruminally-undegradable protein is not degraded by ruminal microorganisms and therefore exits the rumen (Satter and Roffler 1975). However, RDP has the potential to be degraded by ruminal microorganisms to peptides, AA, and NH_3 (Satter and Roffler 1974), as illustrated in Figure 2.1. The Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al. 1992) further characterizes feed protein by partitioning it into different fractions based on its rate of degradation in the rumen (Sniffen et al. 1992). Therefore, the CNCPS provides an estimate of feed protein availability to ruminal microorganisms (Roe et al. 1990). True protein is degraded to peptides and AA and ultimately to NH_3-N (Bach et al. 2005). Non-protein N, consists of N in free ammonia, ammonium salts, urea, nucleic acids, biuret, nitrate, peptides, and free AA. Non-protein nitrogen is highly soluble in the rumen and can be utilized by most ruminal microorganisms (Bach et al. 2005).

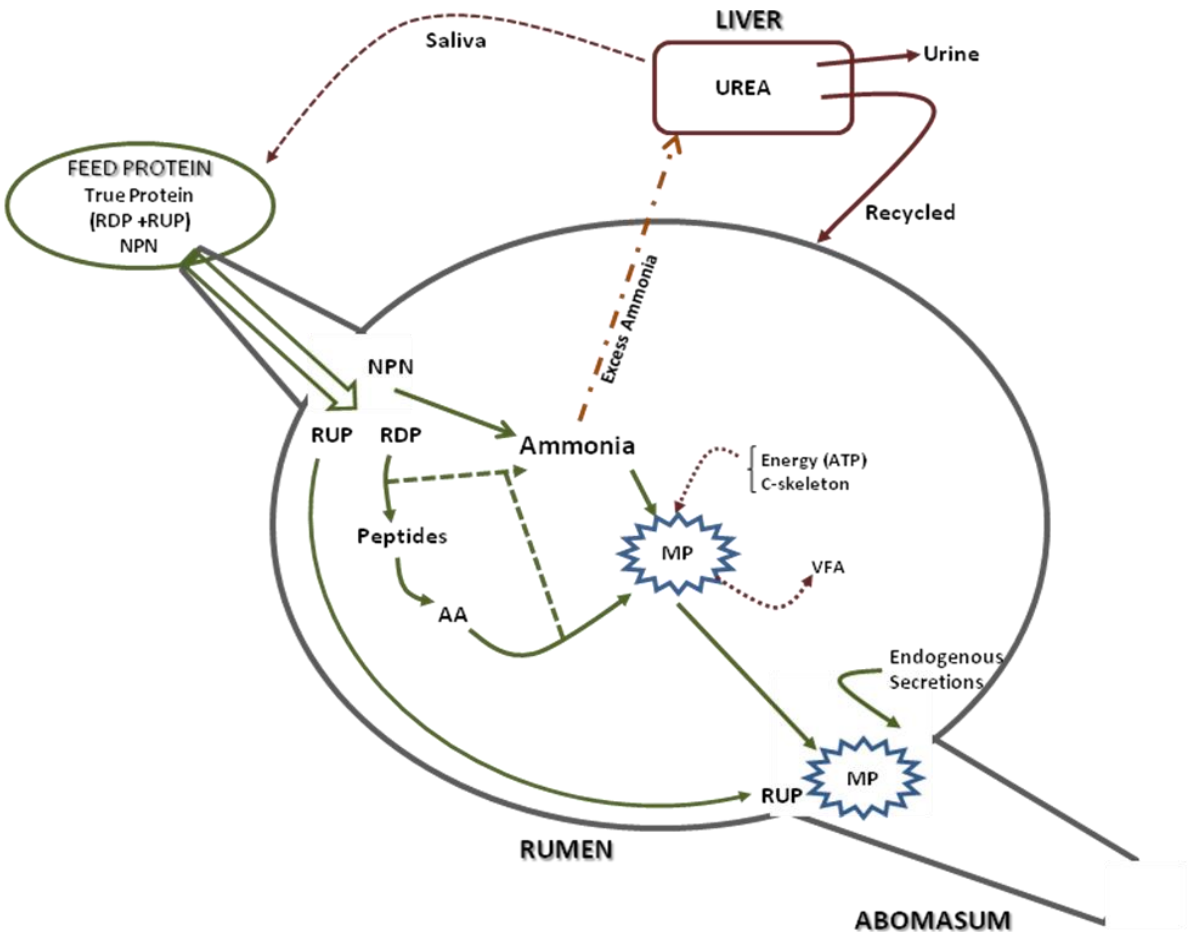

Figure 2.1. Ruminal nitrogen metabolism (adapted from Satter & Roffler 1974). Feed protein is degraded by ruminal microorganisms to peptides, amino acids (AA), and ammonia (NH_3). These substrates can then be utilized by microorganisms for growth and proliferation. If NH_3 is produced in excess of the ruminal microorganisms ability to utilize it (i.e., there is insufficient ruminally-fermentable energy available) then the excess is absorbed from the rumen and converted to urea in the liver. Urea is either transported to the kidney and excreted in the urine or recycled to the gastrointestinal tract (GIT) via saliva or across the GIT epithelium. (RDP, ruminally-degradable protein; RUP, ruminally-undegradable protein; NPN, non-protein nitrogen; AA, amino acids; MP, microbial protein).

Multiple microbial species (including bacteria, protozoa and fungi) are involved in feed degradation with specificity for different chemical components of feed but there is also cross-utilization of substrates and a co-dependence in order to digest feed entering the rumen (Orpin and Joblin 1988; Hobson and Stewart 1997; Huntington and Archibeque 2000). This is particularly the case for cell wall components of fiberous feed (Krause et al. 2003). The ruminal bacterial population is numerous ($10^{10} – 10^{11}$ ml^{-1} of rumen digesta) and makes up roughly 60 to 90% of the total ruminal microbial biomass (Hobson and Stewart 1997). Approximately 80% of ruminal bacteria are associated with the particle phase and play a major role in feed digestion (Stewart et al. 1997). Particle associated bacteria are responsible for 30 to 50% of ruminal protease activity (Prins et al. 1983). However, bacteria closely associated with feed particles are generally cellulolytic bacteria involved with the degradation of plant cell walls (Stewart et al. 1997). Bacteria loosely associated with feed particles have been shown to have higher phosphatase, amylase and protease activity (Stewart et al. 1997). Approximately 20% of ruminal bacteria are associated with the fluid phase and 1 to 2% of total bacteria with the rumen wall (Stewart et al. 1997). Most fluid phase bacteria have become detached from feed particles and subsist on soluble feed components and therefore must constantly seek out soluble substrate (Krause et al. 2003). The rate of cell division of fluid associated bacteria must be greater than that of the ruminal fluid outflow rate to ensure survival (Van Soest 1994). Epimural bacteria are bacteria that adhere to the ruminal epithelium and are mostly facultative anaerobes that sequester oxygen as well as hydrolyze urea entering the rumen (Krause et al. 2003). Epimural bacteria are highly proteolytic and recycle protein from sloughed epithelial cells (Hobson and Stewart 1997).

Ruminal protein degradation can be considered a multi-step process. The first step in protein degradation involves the transport to and colonization of feed particles by ruminal

microbes (Wallace et al. 1997). Secondly, microbial attachment to feed particles is important because it places the substrate and microbial enzymes in close proximity and allows the subsequently released nutrients to be more readily captured by adherent microorganisms (McAllister et al. 1994). The third step is specific adhesion of microorganisms with the substrate.. Both primary and secondary colonizers form a multispecies biofilm. The microorganisms in this biofilm produce an array of enzymes that digest the feed substrate releasing soluble substrates that can be utilized by the microorganisms which then release their end-products of digestion for utilization by other microorganisms (McAllister et al. 1994). This microbial consortium therefore becomes a highly collaborative system resulting in a stable fermentation environment.

In the case of protein, microorganisms, including bacterial species such as *Prevotella spp.*, and *Butyrivibrio fibrisolvens* (Prins et al. 1983) release extracellular proteases, peptidases and deaminases which degrade proteins to peptides, AA and NH_3, respectively (Prins et al. 1983; Wallace et al. 1997). These substances are then taken up intracellularly by ATP dependent transport pathways (Leng and Nolan 1984). If there is sufficient energy available from carbohydrate digestion, then peptides and AA and NH_3-N are incorporated into microbial AA and protein (Bach et al. 2005). It has been shown that at high NH_3 concentrations, N assimilation occurs via a process involving glutamate dehydrogenase (Leng and Nolan 1984). However, under low NH_3 concentrations, NH_3 is fixed in a two-step process involving glutamine synthetase and glutamate synthase (Leng and Nolan 1984). These reactions include the addition of an amide group to glutamate to form glutamine followed by a reductive transfer of the amide-N of glutamine to 2-oxoglutarate which requires ATP (Leng and Nolan 1984). Some species of ruminal bacteria have a preference for NH_3-N as their primary N source (Hungate 1966);

however growth of others has been shown to increase when AA and peptides are present (Wallace 1997).

Conversely, as depicted in Figure 2.1, if there is insufficient energy available, then peptides, AA and NH_3 are unable to be incorporated into microbial protein (Bach et al. 2005). Ruminal bacteria do not have mechanisms to transport AA out of the cell (Tamminga 1979). Therefore, excess AA are deaminated to NH_3, VFA and CO_2 in order to be passed out of the cell into the ruminal environment (Groff and Gropper 2000).

Metabolizable protein (MP) reaching the small intestine represents AA that are available for absorption by the host animal (NRC 2001). Metabolizable protein consists of RUP, endogenous N sources (sloughed epithelial cells, digestive enzymes) as well as microbial protein (Satter and Roffler 1974). Microbial protein is a source of high quality protein (i.e. microbial protein closely matches the AA profile of meat and milk) and accounts for between 60 and 80% of absorbable AA arriving at the small intestine (Storm and Ørskov 1983).

Ruminal protozoa are slower growing than bacteria and are highly affiliated with the particle phase of ruminal digesta in order to prevent their washout from the rumen (Williams and Coleman 1997). With limited passage of protozoa to the lower GIT, they contribute little metabolizable protein to the host animal (Jouany 1996). However, protozoa cannot synthesize AA from simple compounds of N and, therefore, they engulf ruminal bacteria or feed particles (i.e. protein fragments) (Jouany 1996). The synthesis and breakdown of bacterial N by protozoa represents a significant proportion of N recycling in the rumen. In addition, protozoa have been shown to be highly proteolytic (Williams and Coleman 1997). One of the main end-products of protozoa digestion is large amounts of NH_3 (Williams and Coleman 1997). Therefore, protozoa

significantly contribute to the ruminal NH_3 pool, potentially increasing the need for detoxification of NH_3 through the formation of urea, which is an energetically costly process.

2.3 Ammonia Absorption

The ruminal ammonia pool consists of ammonia from microbial degradation of dietary true protein, and from NPN sources such as urea, uric acid, nitrate, and dissolved nucleotides (Satter and Roffler 1974). In addition, ammonia is derived from protozoa metabolism, as well as gaseous N_2 entering the rumen with feed (Bach et al. 2005). Ruminal ammonia can exit the rumen via three major routes; 1) Ammonia can be incorporated into microbial cells as AA which can then pass out of the rumen; 2) ammonia can also flow out of the rumen but the magnitude is dependent on the ruminal fluid outflow rate; and 3) ammonia can be transferred across the ruminal epithelium (Satter and Roffler 1974).

Although ammonia is transferred across the whole GIT, the rumen is the major site of absorption with only 33% of ammonia being absorbed across the small intestine, large intestine, and cecum (Reynolds and Huntington 1988). In addition, Nolan and Leng (1972) estimated that in sheep, 45% of plasma urea-N is derived from ruminal ammonia-N (RAN). Figure 2.2 is a schematic representation of ammonia transfer across the GIT epithelium. Ruminal ammonia is present as both NH_3 (unionized lipid-soluble) and NH_4^+ (ionized less lipid-soluble). The unionized form of ammonia (NH_3) is transferred via passive diffusion across the lipid bilayers of the ruminal wall, diffusing down a concentration gradient to the epithelial cell (Hogan 1961). However, at a ruminal pH of 6 to 7 ammonium (NH_4^+) predeominates, is less lipid-soluble, and is transported via potassium channels in the ruminal epithlium (Bodeker and Kemkowski 1996; Rémond et al, 1996).

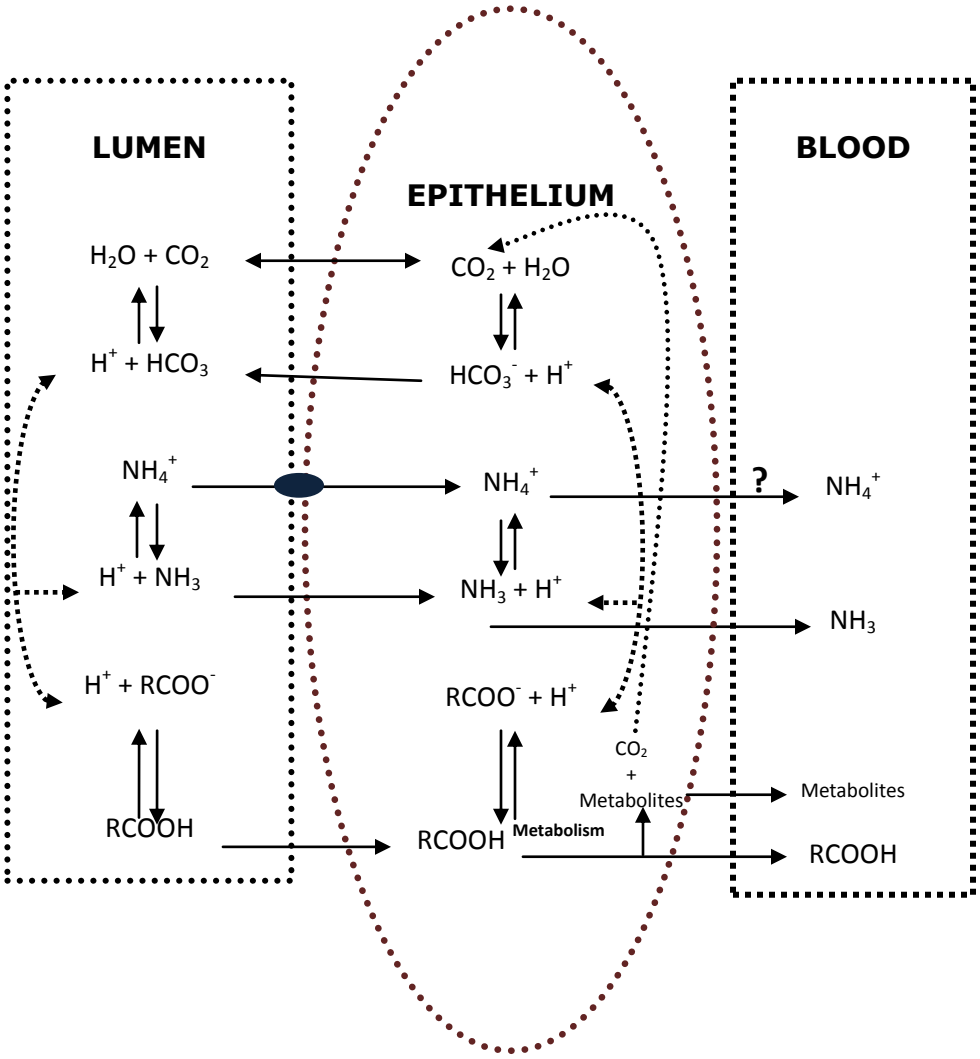

Figure 2.2. Absorption of ammonia across the ruminal epithelium (adapted from Bödeker et al. 1992a,b; Bödeker and Kemkowski 1996; Rémond et al, 1996). The unionized, more lipid soluble form of ammonia (NH_3) diffuses into the epithelial cell via a concentration gradient. However, when ruminal pH is between 6 and 7, ammonium (NH_4^+) is predominate which dissociates into NH_3 and H^+ after absorption. During absorption, NH_3 forms NH_4^+ by incorporating H^+ ions from H_2CO_3 dissociation to HCO_3^- + H^+ as well as from the dissociation of RCOOH (short chain fatty acids) to $RCOO^-$ + H^+. The transport of NH_4^+ across the ruminal epithelium is facilitated by potassium channels (⬬) in the ruminal epithelium.

Both ruminal and dietary factors can influence ammonia transfer from the rumen (Huntington 1986; Reynolds and Huntington 1988; Abdoun et al. 2007; Abdoun et al. 2010). Dietary protein concentration and intake influence ruminal ammonia concentration (Reynolds and Kristensen 2008) as does the forage to concentrate ratio of the diet (Huntington 1986; Reynolds and Huntington 1988). The incorporation of ruminal ammonia into microbial protein is an energy-dependent process. This concept was demonstrated by Reynolds (1996) who reported a reduction in ruminal ammonia concentration after infusion of starch into the rumen. Ruminal pH is the major determinant of the NH_3 to NH_4^+ ratio (Siddons et al. 1985; Abdoun et al. 2007) and therefore dietary alterations influencing ruminal pH affect ammonia transfer from the rumen. The influence of short-chain fatty acids (SCFA) and CO_2 on ammonia transfer have been discussed above and outlined by Bödeker et al. 1992a,b and Abdoun et al. 2010. Intracellular carbonic anhydrase is an enzyme involved in the release of HCO_3^- and H^+ from CO_2 and H_2O where the H^+ can then be used in the formation of NH_4^+ from absorbed NH_3 (Bödeker et al. 1992a). Furthermore, the non-ionized form of SCFA are absorbed into the epithelial cell and then dissociate, releasing H^+ which can be utilized by NH_3 to from NH_4^+ therefore reducing the intracellular NH_3 concentration and facilitating further absorption of NH_3 (Bödeker et al. 1992b).

2.4 The Urea Cycle

Ammonia can become toxic if allowed to accumulate in tissues (> 0.5 mg dL^{-1}). Toxicity symptoms include tremors, blurred vision, coma, irreversible brain damage and even death (> 4.0 mg dL^{-1}) (Singer 2003). Different species deal with this problem in different ways. Aquatic animals are classified as ammonotelic as they excrete ammonia from the gills into the surrounding water where the dilution effect detoxifies the ammonia (Swenson and Reece 1993;

Singer 2003). Birds and terrestrial reptiles are classified as uricotelic. They convert ammonia into uric acid which can be stored in a solid form in a shelled egg or excreted in concentrated urine (Wright 1995; Stewart and Smith 2005). Mammals, on the other hand, have evolved a process in the liver called the ornithine or urea cycle and are classified as ureotelic (Singer 2003). In mammals, ammonia is transported to the liver via the portal blood where it is detoxified by conversion to urea (Stewart and Smith 2005).

The urea cycle consists of a series of metabolic reactions which occur in the mitochondrial matrix and cytosol of the periportal hepatocyte cells of the liver (Haussinger 1983; Haussinger et al. 1992). However, in perivenous hepatocytes, ammonia is utilized for glutamine synthesis, which serves as a scavenging mechanism for ammonia that avoides the formation of urea in periportal hepatocytes (Haussinger et al. 1992). The periportal hepatocytes are the main site of urea synthesis and a series of enzymes are employed to convert ammonia into urea. Briefly (Figure 2.3), ammonia first combines with CO_2 or HCO_3^- in the mitochondrial matrix to form carbamoyl phosphate (Meijer et al. 1990). A carbamoyl group is then added to ornithine, forming citrulline, a reaction which is catalyzed by ornithine transcarbomoylase. Citrulline is translocated into the cytosol where citrulline and aspartate are then combined to form argininosuccinate which is then split into fumarate (which can enter the tricarboxylic acid cycle to serve as an intermediate for aspartate production) and arginine. Arginine is cleaved by hydrolysis to release urea, a reaction which regenerates ornithine. Therefore, ammonia is converted to urea using the cyclically regenerated ornithine as a carrier (Garret and Grisham

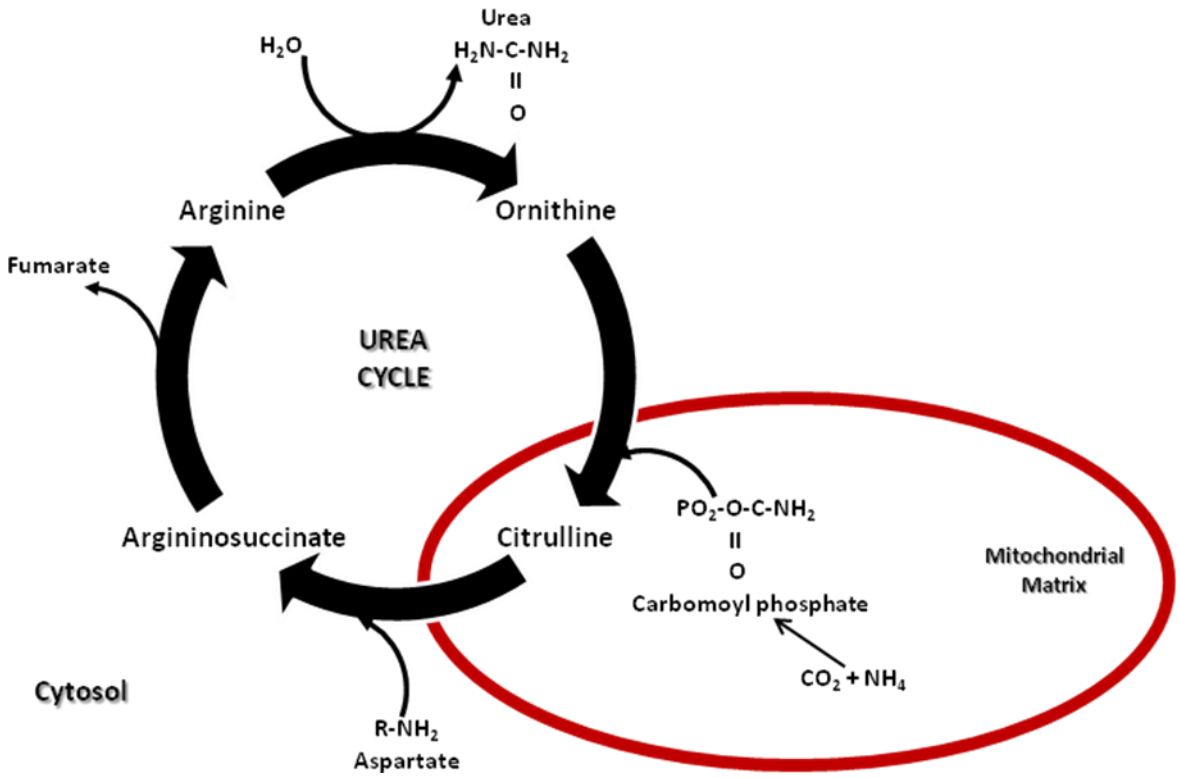

Figure 2.3. Metabolic reactions of the urea cycle (adapted from Takiguchi and Mori 1995; Xie 2003). The urea cycle converts ammonia from amino acid degradation to urea. The first reaction in the urea cycle is the condensation of ornithine and carbamaylphosphate forming citrulline (in the mitochondrial matrix with the enzymes carbomoyl phosphate synthetase and transcarbamoylase). Citrulline is then exported into the cytosol. Citrulline and aspartate then link to form argininosuccinate (enzyme involved: arginosuccinate synthetase) which splits to form fumarate and arginine (enzyme involved: arginosuccinate lyase). Arginine is cleaved by hydrolysis (enzyme involved: arginase), releasing urea and resulting in the cyclic regeneration of ornithine.

1999). Urea formation represents an effective way to detoxify excess ammonia but it is also energetically expensive. Three moles of ATP are required in addition to one molecule of CO_2 and one molecule of aspartate to convert ammonia to urea (Swenson and Reece 1993). This results in one molecule of urea, one of fumarate, two ADP molecules, one molecule of inorganic pyrophosphate (PPi) and two of inorganic phosphorus (Pi) (Swenson and Reece 1993).

Rates of hepatic urea-N production (UER) vary among species and are influenced by several dietary factors. For example, monogastrics, such as humans have low rates of UER (11.3 g N d^{-1}; McClelland and Jackson 1996) as compared to sheep (3-22 g N d^{-1}; Lobley et al. 2000; Sunny et al. 2007; Kiran and Mutsvangwa 2010), beef cattle (20-127 g N d^{-1}; Archibeque et al. 2001; Wickersham et al. 2008a,b), and dairy cows (262-483 g N d^{-1}; Gozho et al. 2008).

2.5 Urea-Nitrogen Recycling

Urea synthesized in the liver is either transported to the kidneys for excretion in the urine or it can be returned to the GIT, a process termed 'urea recycling' (Lapierre and Lobley 2001; Stewart and Smith 2005; Reynolds and Kristensen 2008). Total hepatic urea-N production is often greater than intake of apparently digestible N therefore if no urea-N was returned to the GIT the host would be in a negative N balance (Lapierre and Lobley 2001). Urea-N recycling occurs in both ruminant and non-ruminant animals. However, in ruminants, 40 to 80% of endogenously produced urea-N can be recycled to the GIT as compared to 15 to 39% in non-ruminants (Huntington 1989; McClelland and Jackson 1996; Russell et al. 2000). In ruminants, urea-N can be recycled to the GIT via transfer from the blood to the lumen of the GIT (Houpt and Houpt 1968; Ritzhaupt et al. 1998; Stewart et al. 2005). However, it has been estimated that 23 to 69% of endogenously produced urea-N is recycled to the GIT through the saliva in

ruminants (Huntington 1989). The recycling of urea-N to the GIT represents an opportunity for the anabolic use of recycled urea-N, improved overall N efficiency and the opportunity to reduce the excretion of N into the environment (mostly via urinary urea-N; UUN).

2.6 Urea-Nitrogen Transfer to the Gastrointestinal Tract

Urea-N can be recycled across the epithelial lining of all compartments of the GIT, including the rumen, small intestine and large intestine (Varady et al. 1979). Lapierre and Lobley (2001) reported that 70% of endogenously produced urea-N enters the GIT by recycling to the small intestine and limited amounts are recycled to the large intestine (cecum and colon). The rate of entry into the small intestine varies depending on the diet fed. For example, when beef steers were fed a high concentrate diet, only 19% of urea-N entered the small intestine (Reynolds and Huntington 1988) but this increased to 90% when steers were fed high fiber diets (Huntington 1989). Urea-N entering the large intestine is utilized for microbial protein synthesis, especially when fermentable energy sources are this site are readily avaialble (Kennedy and Milligan 1980); however, microbial protein produced at this site is excreted in the faeces and therefore does not contribute to the AA or anabolic processes of the host (Lapierre and Lobley, 2001). Therefore, increasing the proportion of urea-N recycled to the rumen is preferable in terms of incorporation of recycled urea-N into microbial protein and improving the efficiency of N utilization in ruminants.

2.6.1 Urea-Nitrogen Transfer into the Rumen

The rate of urea-N entry into the rumen has been reported to vary from 27 to 60% of urea-N entering the GIT (Houpt 1970; Egan et al. 1986; Kennedy and Milligan 1980). Harmeyer

and Martens (1980) estimated that between 40 and 80% of endogenous urea-N production entered the GIT, but this is substantially influenced by diet. For example, Huntington (1989) reported a greater percentage of urea-N entering the rumen (as a proportion of urea-N entry to the GIT) in steers fed a high concentrate diet as compared to those fed a low concentrate diet (i.e. 95 vs. 62.5%, respectively). The amount of urea-N entering the GIT via saliva is dependent on the amount of saliva produced and blood urea-N concentration. Saliva production can be quite large in cattle (220-250 liters d^{-1}; Maekawa et al. 2002) but can vary depending on the physical form of the diet. For instance, high roughage diets stimulate greater saliva production than high grain diets (Huntington 1989; Theurer et al. 2002). Huntington (1989) reported 69% of urea-N in the rumen originated from saliva in forage-fed cattle as compared to only 23% in concentrate-fed cattle. Therefore, increasing the forage content of the diet can increase saliva production and the proportion of urea-N recycled to the GIT via saliva.

The second route for recycling of urea-N is across the rumen wall via the blood. Previous studies have shown that urea crosses the ruminal epithelium primarily by passive diffusion down a blood-rumen urea concentration gradient (Houpt and Houpt 1968). Bacteria colonizing the ruminal epithelium (epimural bacteria; Wallace et al. 1997) utilize urease to hydrolyze incoming urea to NH_3 and carbon dioxide (CO_2) (Reynolds and Kristensen 2008), thereby maintaining the concentration gradient to allow diffusion of urea into the rumen. It has been shown that urease activity decreases as ruminal NH_3 concentration increases (Cheng and Wallace 1979). Furthermore, Marini et al. (2004) reported a linear decrease in urease activity with increasing levels of N intake which led to an increase in ruminal NH_3 concentrations. Therefore, ruminal NH_3 concentration and bacterial urease activity are thought to be important factors in the recycling of urea-N to the rumen (Kennedy and Milligan 1978).

It has also been suggested that urea recycling to the rumen is also influenced by the permeability of the ruminal epithelium (Harmeyer and Martens 1980). Further studies have revealed the presence of bidirectional facilitative urea transporter (UT) proteins in the ruminal epithelium of both sheep and cattle (Ritzhaupt et al. 1997, 1998; Marini and van Amburgh 2003) and the bovine UT (UT-B) structure has been determined (Stewart et al. 2005). The expression of UT-B gene could influence the rate of urea-N recycling. The expression of UT-B has been found to depend on dietary N content in cattle (Marini and van Amburgh 2003), but this relationship has not been substantiated in sheep (Marini et al. 2004). Other transporters of urea include the large family of water transporters called aquaporins (AQP) which have been found in several different tissues such as the GIT (Ma and Verkman 1999), including the bovine ruminal papillae (Røjen et al. 2011). Røjen et al. (2011) found that AQP 3, 7, and 8 were expressed in the ruiminal epithelia of lactating dairy cows but were not correlated with increased permeability of the ruminal epithelial to urea on low N diets. It was concluded that AQP do not play a role in the regulation of ruminal epithelial urea transport.

Research has shown that between 46 to 63% of urea-N recycled to the GIT can be utilized for anabolic purposes (Sarrasecca et al. 1998; Archibeque et al. 2001; Lobley et al. 2000). Furthermore, Wickersham et al (2008a, b) reported that 72% of urea-N recycled to the GIT was incorporated into microbial protein in cattle or sheep fed low CP diets. This mechanism is important because it helps to conserve N and counteracts the development of a negative N balance when dietary N supply is limited or intermittent. Urea-N recycled to the rumen can contribute to microbial protein synthesis which supplies between 60 and 80% of metabolizable protein at the small intestine (Satter and Roffler 1974). This is important because microbial

protein is a high quality protein with an excellent AA profile compared to that of meat and milk (Ørskov 1992).

2.7 Factors Affecting Urea-Nitrogen Recycling to the Rumen

The rate of urea-N recycled to the rumen and its utilization for anabolic purposes can be influenced by a number of both dietary and ruminal factors. Many ruminal and dietary factors are interrelated in terms of affecting urea-N recycling to the rumen. For example, the recycling of urea-N to the rumen has been shown to be negatively correlated with ruminal NH_3 concentration (Kennedy and Milligan 1980). Therefore, dietary factors affecting the rate of dietary N partitioning to ammoniagenesis will influence urea-N recycling to the rumen (Lapierre and Lobley 2001). Furthermore, increasing the supply of ruminally-fermentable carbohydrate increases the incorporation of ruminal NH_3-N into microbial protein. This, in turn, reduces ruminal NH_3-N concentration leading to an increase in the proportion of urea-N recycled to the rumen (Kennedy and Milligan, 1980). These and other factors are discussed in more detail below.

2.7.1 Dietary Nitrogen Intake

There is consistent evidence showing that decreasing dietary CP level decreases plasma urea-N concentration (PUN), the production of endogenous urea, the absolute amount of urea-N recycled to the ruminant GIT (Kennedy and Milligan 1980; Marini and Van Amburgh 2003; Reynolds and Kristensen 2008; Huntington et al. 2009; Muscher et al. 2010) and results in a decrease in the amount of urea excreted in the urine (Bunting et al. 1987; Archibeque et al. 2002). Lobley et al. (2000) showed that total endogenous urea-N production (as a percentage of

N intake) can vary from 77 to 95% and that this variation is, in part due to changes in dietary N intake. Archibeque et al. (2001) found that endogenous urea-N recycled to the GIT was 11.5% lower when steers were fed forage grown under a high N fertilization rate than those grown with a lower level of N. In addition, Marini et al. (2004) showed that a decrease in dietary N intake resulted in a linear decrease in urea-N recycled to the GIT. However, as a percentage of dietary N intake, the amount of urea-N recycled to the GIT was greater for sheep fed low N diets as compared to those fed high N diets. This mechanism allows ruminants to survive under low or deficient dietary N conditions due to the urea salvage mechanism of urea-N recycling. Marini and Van Amburgh (2003) found that, as a proportion of total endogenous urea-N production, urea-N recycled to the GIT ranged from 29 to 42% in heifers fed dietary N concentrations from 34.0 to 25.0 g N kg^{-1} of DM. Furthermore, and of importance to this thesis is the fact that as a proportion of endogenously produced urea-N, ruminants fed low N diets used a greater quantity of recycled urea-N for productive purposes (i.e. microbial protein production) than ruminants fed high N diets (Bunting et al. 1989). Also, studies in lambs (Bunting et al. 1987) demonstrated that N flow at the duodenum was 16% greater than N intake for lambs consuming a low protein (12 g N/d) diet. The authors suggest that N flow at the duodenum in excess of N intake could represent either a net flow of endogenous protein or the recycling of urea-N to the rumen.

2.7.2 Dietary Ruminally-Degradable Protein Concentration

Dietary feed ingredients with a higher level of RDP result in higher ruminal NH_3-N concentration which is negatively correlated with urea-N recycling across the ruminal epithelium (Kennedy and Milligan 1980) and is therefore an important factor influencing urea-N recycling in ruminants. In addition, ruminal NH_3-N concentration is negatively related to urease activity

(Cheng and Wallace 1979) and the subsequent recycling of urea-N to the rumen (Kennedy and Milligan 1980). As the level of RDP increased for steers fed low quality forage, there was an increase in endogenously produced urea-N and its subsequent recycling to the rumen (Wickersham et al. 2008a). In addition, Wickersham et al. (2009) found that increasing RDP level in steers fed a low dietary N level, resulted in a linear increase in urea-N recycled to the rumen and its incorporation into microbial protein. Rémond et al. (2009) showed that ruminal ammonia loss was reduced for sheep fed a low RDP diet (extruded peas) as compared to sheep fed a high RDP diet (raw peas), resulting in an increase in efficiency of urea-N recycled to the GIT. This observation is supported when Siddons et al. (1985) fed grass silage or dried grass hay to sheep and observed that this resulted in a net loss of 4.0 g N d^{-1} between the mouth and the duodenum with silage as compared to a net gain of 5.5 g N d^{-1} with grass hay. The explanation for this observation was that more silage NPN was degraded in the rumen leading to a higher ruminal NH_3-N concentration limiting the entry of urea-N into the rumen (Siddons et al. 1985). Furthermore, Brake et al. (2010) altered N supplementation (urea vs. DDGS) in steers consuming corn-based diets and found that, as a proportion of total microbial N, microbial capture of recycled urea-N tended to be greater for the DDGS diet than the urea diet. This demonstrates a greater reliance of ruminal microbes on recycled urea-N at a higher dietary RUP content.

2.7.3 Dietary Ruminally-Fermentable Carbohydrate Concentration

Research has demonstrated that mechanical processing of cereal grains can partially shift the site of starch digestion from post-ruminal compartments to the rumen (Theurer et al. 1999; Huntington 1997). Increasing dietary ruminally-fermentable carbohydrate level has been shown to increase urea-N recycling to the rumen (Kennedy 1980; Kennedy and Milligan 1980;

Huntington 1989). For example, Huntington (1989) found that steers fed high concentrate diets recycled 45% of endogenously produced urea-N in the rumen but only 7% was recycled to the rumen of steers fed an alfalfa diet. Furthermore, Theurer et al. (2002) showed that shifting the site of starch digestion from the small intestine to the rumen by feeding steam-flaked as compared to dry-rolled sorghum grain resulted in a 30% increase in urea-N recycling to the rumen in beef steers. Lobley et al. (2000) fed an all forage diet (50% grass hay pellets and 50% dry chopped hay) and mixed concentrate and forage diet (50% hay, 30% barley, and 20% supplement) to sheep and found that on the concentrate and forage diet both endogenous urea-N production and urea-N recycled to the GIT increased. Huntington et al. (2009) also demonstrated that increasing the rumially-degradable carbohydrate level fed to steers on a predominantly forage diet led to an increase in urea-N utilized for anabolic purposes. The greater recycling of urea-N to the GIT could be attributed to greater incorporation of ruminal NH_3-N into microbial protein which would decrease the ruminal NH_3-N concentration, in turn increasing urea-N recycling to the rumen (Kennedy and Milligan 1980) and decreasing N excretion (Huntington 1997; Theurer et al. 1999).

The effect of dietary energy and protein supplementation on urea kinetics was studied in growing beef steers fed prairie hay (Bailey et al. 2012a). In growing steers, energy and protein suppplemetation did not affect urea-N entry rate or gut entry of urea-N. However, increasing casein supplementation (120 g/d vs. 240 g/d dosed ruminally) reduced the amount of microbial N derived from recycled urea-N. This data shows there is opportunity to manipulate the microbial capture of recycled urea-N to improve the efficiency of N utilization in ruminants. However, further research is required to further elucidate the effect of combining different dietary factors

(i.e., RDP and ruminally-fermentable carbohydrate level) on urea-N recycling and microbial protein production in ruminants.

2.7.4 Ruminal NH_3-N Concentration

It has been consistently shown that an increase in dietary CP level results in an increase in ruminal NH_3-N concentration (Cunningham et al. 1996; Kebreab et al. 2002; Reynal and Broderick 2005; Kiran and Mutsvangwa 2010) as well as a decrease in ruminal bacterial urease activity (Marini et al. 2004). In addition, Cheng and Wallace (1979) demonstrated that an increase in ruminal NH_3-N concentration reduced ruminal urease activity. This is of significance because bacterial urease is responsible for hydrolysis of urea-N to NH_3 and CO_2 and thereby maintains a concentration gradient which favors the transfer of urea-N across the ruminal epithelium (Rémond et al. 1996). Houpt and Houpt (1968) reported a decrease in urea-N transfer as urease activity decreased. Kennedy and Milligan (1980) demonstrated that urea-N transfer to the rumen decreased at high ruminal NH_3-N concentrations. Therefore, ruminal NH_3-N concentration can directly affect the transfer of urea-N to the rumen and is an important factor influencing urea-N recycling to the GIT. In cattle, a ruminal NH_3-N concentration of 5 to 8 mg dL^{-1} results in maximal ruminal epithelial transfer of urea-N (Kennedy and Milligan 1978). Therefore, it is important to understand how dietary factors can influence ruminal NH_3-N concentration and the subsequent transfer and utilization of urea-N in the GIT.

As previously discussed, changing RDP level can be used to manipulate ruminal NH_3-N concentration and the rate of urea-N recycling to the GIT. However, protozoa are also important ruminal microorganisms comprising 20 to 70% of the ruminal biomass (Jouany 1996) as well as in terms of intra-ruminal recycling of N. For example, between 10 and 40% of total ruminal N is

sequestered in protozoa (Williams and Coleman 1997). Protozoa are highly proteolytic, ingesting and degrading bacteria and releasing NH_3 into the ruminal environment (Jouany 1996). Defaunation is the removal of ruminal protozoa from the rumen by means such as the application of substances that are toxic to protozoa (e.g. copper sulphate or canola and sunflower oil), or by isolating animals at birth or by emptying and sterilization of ruminal contents (Jouany 1996). Defaunation results in a reduction in ruminal NH_3 concentrations (Jouany 1996; Ivan et al. 2001) and has been shown to increase the amount of bacterial protein flowing to the duodenum (Koenig et al. 2000). Therefore, depending on protein concentration in the diet, defaunation can increase the recycling of urea-N to the rumen (Kiran and Mutsvangwa 2010).

2.7.5 Ruminal Volatile Fatty Acid Concentration and Ruminal pH

Volatile fatty acids (i.e., acetate, propionate and butyrate) are byproducts of ruminal microbial metabolism which are released into the ruminal environment. As ruminal VFA concentration increases, ruminal pH declines (Pylot et al. 1999), making these two factors closely related. In terms of urea-N transfer to the rumen, ruminal butyrate concentration has been shown to positively affect urea-N transfer to the rumen (Thorlacius et al. 1971; Engelhardt et al. 1978). It was suggested that changes in ruminal concentration of VFA influences the permeability of the ruminal epithelium to urea-N (Harmeyer and Martens 1980). More recently, Simmons et al. (2009) fed steers either a concentrate- or silage-based diet leading to a numerically greater butyrate concentration for the concentrate-fed steers. Furthermore, their results showed a higher expression of UT-B mRNA for steers fed the concentrate as compared to the silage-based diet, demonstrating a possible link between ruminal butyrate concentration and UT-B expression.. The rumen is a highly reduced environment leading to the conversion of NH_3

to NH_4^+ and increasing ruminal concentration of NH_4^+ which results in a decrease in ruminal urease activity (Marini et al. 2004) in turn reducing urea-N transfer to the rumen (Rémond et al. 1993; 1996). Furthermore, Abdoun et al. (2010) observed, using isolated ruminal epithleia mounted in Ussing chambers that a reduction of ruminal mucosal buffer pH from 7.4 to 5.4 (and in the presence of short chain fatty acids) resulted in a bell-shaped relationship with mucosal pH. Maximal urea flux was observed around pH 6.2 and as pH dropped below 5.8 urea flux decreased to prestimulation levels (Abdoun et al. 2010). Therefore short-term changes in urea flux may be mediated by changes in mucosal pH. Altering certain ruminal factors, such as an increase or decarase in ruminal pH, can have a positive influence on urea-N transfer across the ruminal epithelium (Abdoun et al. 2010). However, in vivo observations in Holstein steers demonstrated that changes in pH were not accompanied by a reduction in urea-N recycling as was expected (Titgemeyer et al. 2012). These researchers reported that other factors may have inhibited urea-N transport. There is a need for more research to further elucidate the many potential factors influencing urea-N transport across the ruminal epithelium.

2.7.6 Plasma Urea-Nitrogen Concentration

Plasma urea-N (PUN) can be recycled to the rumen across the epithelial tissue via simple passive diffusion (Houpt 1959) and be hydrolyzed by bacterial urease to NH_3 for subsequent utilization for AA synthesis by ruminal microorganisms (Houpt 1959). Previous research has shown that the transfer of urea to the GIT is positively correlated to PUN concentration (Vercoe 1969; Harmeyer and Martens 1980; Huntington and Archibeque 2000). Sunny et al. (2007) fed sheep a low protein diet (6.8% CP on DM basis) and infused urea at different rates (0, 3.8, 7.5 or 11.3 g urea N d^{-1}) into the jugular vein. As PUN concentration increased so did the amount of

urea-N entering the GIT; however, the increase was less as PUN concentration became greater. This is further evidence of the relationship between PUN concentration and urea-N recycling to the GIT. Earlier, Lapierre and Lobley (2001) gathered data from studies in cattle showing no evidence linking PUN concentration and urea-N recycling to the GIT. However, several other factors, such as ruminal concentrations of CO_2, butyrate and ammonia, may have influenced the rate of urea-N recycling to the rumen and counteracted the effects of PUN concentration.

2.8 Conclusions

Inefficient utilization of dietary N can be detrimental to animal production systems and the environment. The excretion of excess dietary N can lead to pollution of the environment and an increase in feeding costs in ruminant production systems. Several dietary strategies have been investigated to address these issues. For example, reducing dietary N intake and increasing microbial capture of recycled urea-N can both lead to a decrease in the excretion of N and improve the overall efficiency of N utilization. Investigating how simultaneous changes in different dietary factors affect urea-N recycling in ruminants may provide further insight into strategies to improve N efficiency.

2.9 Hypothesis and Objectives

It was hypothesized that the efficiency of nitrogen utilization can be improved by enhancing urea-nitrogen recycling to the rumen through the judicious balance of ruminal fermentable carbohydrate and the level and ruminal degradability of dietary protein.

The objectives of the research carried out in this thesis were to determine the effects of:

a) Feeding diets containing two levels of dietary CP (10.8 vs. 14.0%) and RDP (76.0 vs. 73.6% of CP) on urea-N transfer to the GIT, N balance, and microbial protein production in beef heifers;

b) Feeding diets containing two levels of RDS (28.6 vs. 69.2% of total starch) and RDP (48.0% vs. 55.0% of CP) on urea-N recycling to the GIT, N balance, duodenal nutrient flow, and microbial protein production in beef heifers fed low CP diets; and

c) Feeding diets containing two levels of dietary CP (14.9 vs. 17.5%) and RDP (63.0 vs. 69.0% of CP) on urea-N recycling, microbial protein production, N balance, omasal nutrient flow, and milk production in lactating dairy cows.

3 EFFECTS OF DIETARY CRUDE PROTEIN AND RUMINALLY-DEGRADABLE PROTEIN LEVELS ON UREA RECYCLING, MICROBIAL PROTEIN PRODUCTION, AND NITROGEN BALANCE IN BEEF HEIFERS

3.1 Abstract

The objective of this study was to determine the effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) level on urea recycling, microbial protein production and nitrogen (N) balance in beef heifers. Four ruminally-cannulated beef heifers (437 ± 24 kg BW) were used in a 4 × 4 Latin Square design with a 2 × 2 factorial arrangement of dietary treatments with 23 d periods. Jugular infusions of [$^{15}N^{15}N$]-urea (220 mg d^{-1}; 98+ atom percent) were conducted for 4 d (d 18-22) to estimate urea kinetics, with total collection of faeces and urine. Proportions of [$^{15}N^{15}N$]-, and [$^{14}N^{15}N$]-urea in urinary urea, and ^{15}N enrichment in faeces were used to calculate urea kinetics. Microbial N production was estimated using urinary excretion of purine derivatives. Dietary treatments were 10.8 vs. 14.0% CP and 73.4 vs. 76.0% RDP (% of CP). Feeding the low CP diet decreased N intake ($P < 0.01$), ruminal ammonia-N concentration ($P < 0.01$) and urinary N excretion ($P < 0.01$). Endogenous urea-N production increased ($P = 0.03$) with increasing dietary CP level, as did urinary urea-N loss ($P = 0.04$). However, urea-N transfer to the gastro-intestinal tract was similar across diets, with most of this N returned to the ornithine cycle. Microbial N supply was unaffected ($P > 0.05$) by dietary treatment. Therefore, low CP diets showed greater efficiency of N utilization, and less N excreted into the environment with no significant change in microbial protein production.

3.2 Introduction

Dietary protein is extensively degraded in the rumen to peptides, amino acids, and ammonia (NH_3), due to the proteolytic activity of ruminal microorganisms (Prins et al. 1983). However, NH_3-N in excess of ruminal microbial requirement is absorbed across the ruminal epithelium into the portal blood, converted to urea in the liver and excreted via the urine (Lobley et al. 1995). This can be detrimental to the environment (Cowling and Galloway 2002; Klopfenstein and Erickson 2002; Hristov et al. 2011) as well as represent an irreversible loss of nitrogen (N) to the animal. Lowering dietary crude protein (CP) concentration has been found to reduce ruminal NH_3-N concentration in beef steers (Brake et al. 2010). Lowering ruminal NH_3-N concentration enhances the transfer of urea to the rumen (Kennedy and Milligan 1980) through an increase in bacterial urease activity of epimural bacteria (Cheng and Wallace 1979). Bacterial urease aids in the transfer of urea-N across the ruminal epithelium by maintaining a favourable blood-rumen urea-N concentration gradient (Rémond et al. 1996). There is also evidence that other factors such as urea transporters, ruminal pH and VFA concentration influence the recycling of urea-N to the rumen and therefore should be considered (Abdoun et al. 2007; Abdoun et al. 2010). The recycling of urea is an important N salvage mechanism (Stewart and Smith 2005) and can be a significant source of N for microbial growth (Lapierre and Lobley 2001).

Dietary CP consists of both ruminally-degradable (RDP) and ruminally-undegradable (RUP) protein. The peptides, amino acids, and ammonia of RDP are precursors for bacterial protein synthesis (Bach et al. 2005). The amounts of dietary CP and RDP therefore directly influence the availability of these substrates for bacterial protein synthesis (Hristov and Jouany 2005). Feeding a higher level of dietary RDP results in higher ruminal NH_3-N concentration

which is negatively correlated with urea-N transfer across the ruminal epithelium (Kennedy and Milligan 1980). It has been demonstrated that in steers fed low quality forage, increasing dietary RDP level increases both total endogenous urea-N production and urea-N recycling to the GIT (Wickersham et al. 2008a). Furthermore, Wickersham et al. (2009) observed a linear increase in urea-N recycled to the GIT and its incorporation into microbial protein with increasing RDP level in steers fed a low level of dietary N. There is therefore opportunity to improve the efficiency of N utilization through concomitant changes in dietary CP and RDP to influence urea-N kinetics and microbial protein synthesis in ruminants. The hypothesis of this study was that lowering the level of both dietary CP and RDP would result in greater transfer of endogenous urea-N to the gastro-intestinal tract (GIT), thus maintaining microbial protein production. The objective was to determine the effect of varying dietary CP and RDP levels on urea transfer to the GIT, microbial protein production, N balance, and duodenal nutrient flow in beef heifers.

3.3 Materials and Methods

Heifers used in this study were cared for in accordance with guidelines of the Canadian Council of Animal Care (1993) and their use was approved by the University of Saskatchewan Animal Care Committee (UCACS Protocol No. 20040048).

3.3.1 Animals and Experimental Design

Four Speckle Park beef heifers (437 ± 24 kg BW) surgically fitted with ruminal (10 cm diameter opening, Bar Diamond, Parma, ID) cannulas were used in a 4 × 4 Latin square design experiment with a 2 × 2 factorial arrangement of treatments with 23 d periods. Each

experimental period consisted of 18 d of dietary adaptation and 5 d of sample collection. Heifers were housed in individual 3 m × 3 m pens on rubber mats in the Livestock Research Building of the Department of Animal and Poultry Science (University of Saskatchewan). Four isoenergetic diets delivered 2 levels of CP (10.8% vs. 14.0%, DM basis) with 2 levels of RDP (73.4 vs. 76.0% of CP). Canola meal and/or heated canola meal were included in diets to control the RDP level. The canola meal was obtained from Federated Co-Operative Ltd (Saskatoon, SK, Canada) and the heated canola meal (Alberta Gold) from Canbra Foods Ltd (Lethbridge, AB, Canada). Experimental diets were fed twice daily at 0800 and 1600 h as a pelleted ration and heifers had free access to water. Chemical composition of ingredients is presented in Table 3.1. Ingredients and chemical composition of experimental diets are shown in Table 3.2.

3.3.2 Sample Collection

During the 5-d collection period (d 18 to d 23), individual heifer feed intake was recorded daily. Also, samples of total mixed rations (TMR) and orts were collected daily, stored at -20^{o}C and then composited by treatment for each experimental period.

On d 17 of each experimental period, heifers were fitted with temporary vinyl catheters (0.86 mm i.d. × 1.32 mm o.d.; Scientific Commodities Inc., Lake Havasu City, AZ) in both the right and left jugular veins to allow for simultaneous blood sampling and stable isotope infusion. Total N balance (d 18 to d 23) and urea-N transfer to the GIT (d 18 to d 22) were determined using the procedures of Lobley et al. (2000), except that urine was collected using bladder catheters. During total collection of urine and faeces, heifers were restrained within pens. On d 17, indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) were inserted into heifers as described by Crutchfield (1968). Just

before 0800 h on d 18, background samples of urine and faeces were collected and the bladder catheters were connected to urine collection tubing. Starting at 0800 h on d 18, double-labeled urea ([$^{15}N^{15}N$]-urea, 98+ atom percent; Cambridge Isotope Laboratories, MA, USA) in 2 L of sterile saline solution was continuously infused into the jugular vein at a rate of 220 mg d^{-1} using a peristaltic pump (Watson and Marlow, Cornwall, UK. Model: 205U) for 96 h. Urine was collected into 20-L Carboy polyethylene containers into which 80 mL of concentrated HCl (VWR Scientific, Mississauga, ON) had been added in order to maintain a pH < 3. Total daily urine output for each heifer was weighed, mixed thoroughly and a sample (20% of total output) collected. A 50-mL subsample of urine was collected from the composited daily output. In addition, a 2-mL subsample of urine was collected daily from the composited daily output and diluted with 8 mL of distilled water. All urine samples were stored at -20^{o}C. Total faecal output was collected daily for a 5 d period (d 18 to d 23) by thoroughly scraping feces from the pen floors. A sub sample (10% of total output) of feces was taken each day and composited by heifer within each period and stored at -20°C.

Ruminal (every 2 h) and blood (every 4 h) samples were collected over 24 h (0800 h on d 21 to 0600 h on d 22). Ruminal contents (1 L; 250 mL from the cranial ventral, caudal ventral, central, and cranial dorsal regions) were collected, mixed thoroughly and strained through 4 layers of cheesecloth. Ruminal fluid pH was immediately determined on the filtrate, using a Model 265A portal pH meter (Orion Research Inc., Beverly, MA). A 5-mL aliquot of ruminal fluid was preserved with 1 mL of meta-phosphoric acid (25% wt vol^{-1}), and a second 5-mL aliquot was preserved with 1 mL of 1% sulfuric acid and stored at -20^{o}C. A third 15-mL aliquot was not acidified and stored at -20^{o}C for later determination of osmolality. Blood samples (5 mL) were collected from a jugular catheter into tubes containing heparin (144 USP units; Becton

Dickinson, Rutherford, NJ) and transferred to the laboratory on ice. Blood samples were centrifuged at 1,500 × *g* for 15 min at 4°C (Beckman Instruments Inc., Model J6-MC Centrifuge, Mississauga, Ontario, Canada) and the resulting plasma stored at -20°C.

Contents of RDP in the TMR and dietary ingredients were determined using the in situ incubation technique (Yu et al. 2003) with one beef heifer (454 kg BW) that was fitted with a ruminal cannula (10 cm diameter opening, Bar Diamond, Parma, ID). The heifer was housed in a 3 × 3 m pen in the Livestock Research Building (University of Saskatchewan) and was fed a blend of all four experimental diets at 2% of body weight (DM basis) at 0800 and 1600 h daily. Briefly, samples of each dietary ingredient were ground through a 3 mm screen (Christy and Norris Ltd., Chelmsford, England), and then a 7 g sample was weighed into number-coded bags (10 × 20 cm, Nitex 03-41/31 monofilamnet open mesh fabric, Screentec Corp., Mississauga, ON; with a 40 μm pore size). Incubations were carried out for 48, 24, 12, 8, 2, and 0 h. Procedures for ruminal incubation and post-incubation procedures were as described by Yu et al. (2004).

3.3.3 Sample Analyses

After completion of the experiment, frozen TMR, orts and faecal samples were thawed at room temperature and then dried at 55°C for 96 h. Dried TMR, orts, and faecal samples were ground through a 1 mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Samples were then analyzed for DM by oven-drying at 135°C for 2 h [Association of Official Analytical Chemists (AOAC) 1990; method 930.15], OM by ashing at 600°C for at least 8 h (AOAC 1990; method 942.05), CP using the macro-Kjeldahl procedure (AOAC 1990; method 984.13), ether extract (AOAC 1990; method 920.39), acid detergent fiber (ADF) (AOAC 1990; method 973.18), and neutral detergent fiber (NDF; Van Soest et al. 1991). Amylase and

sodium sulfite were used for NDF determination. Dried TMR were analyzed for acid detergent insoluble (ADIN) and neutral detergent insoluble (NDIN) nitrogen (Licitra et al. 1996). Prior to chemical analysis, the dried residues from in situ incubations were ground through a 1 mm screen using a Retch ZM 100 grinder (F-Kurt Retsch GmbH & Co. Kg, Germany) and then analyzed for DM as previously described, and CP using the Dumas method of combustion on a LECO FP-528 Nitrogen/Protein Determinator (AOAC 1990; method 992.23).

Urinary urea-N was determined using the diacetyl monoxime method (Procedure No. 0580, Stanbio Laboratory, Boerne, TX) on background and daily composite 50-mL subsamples of urine prior to processing for the determination of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea enrichments. Urinary urea was then isolated by applying urine containing 1.5 mg of urea-N to a pre-packed cation exchange resin column (AG-50W- × 8 Resin, 100-200 mesh, H+ form; BioRad Laboratories, Hercules, CA) as described by Archibeque et al. (2001). Samples were then eluted with N-free water, air-dried and transferred to borosilicate glass tubes for freeze-drying and then analyzed for the proportions of [$^{15}N^{15}N$]-, and [$^{14}N^{15}N$]-urea in urinary urea by isotope ratio-mass spectrometry (^{15}N Analysis Laboratory, University of Illinois at Urbana-Champaign). The results were corrected for [$^{14}N^{15}N$]-urea produced by non-monomolecular reactions (Lobley et al. 2000). Total N in samples of daily urine output was determined using the macro-Kjeldahl procedure (AOAC 1990; method 976.05). Diluted daily urine samples were composited (proportionally based on daily urine output) by heifer for each period and analyzed for urinary urea-N (d 19 to d 22) using the colorimetric diacetyl monoxime method (Procedure No. 0580, Stanbio Laboratory, Boerne, TX), and for uric acid (Fossati et al. 1980) and allantoin for samples collected on d 26 and 30 (Chen and Gomes 1992).

Frozen plasma samples were thawed and composited by heifer for each experimental period. Urea-N was determined in urine and plasma using the diacetyl monoxime method. Ruminal fluid samples preserved with meta-phosphoric acid were analyzed for volatile fatty acid (VFA) concentration (Erwin et al. 1961) using an Agilent 6890 Series Gas Chromatography system (Wilmington, DE). Ruminal fluid samples preserved with sulphuric acid were analyzed for NH_3-N using a phenol-hypochlorite assay (Broderick and Kang 1980). Osmolality was measured on non-acidified ruminal fluid samples using a Vapro^{TM} Vapor Pressure Osmomemter (Model 5520; Wescor Inc., Logan, Utah).

3.3.4 Calculations and Statistical Analysis

Total N retention was calculated as intake N – faecal N – urinary N. The urinary excretion of purine derivatives (i.e., uric acid and allantoin) was used to estimate microbial nonammonia-N (NAN) production according to the methods of Chen and Gomes 1992. Urea-N kinetics was calculated according to the model of Lobley et al. (2000), using urinary ^{15}N enrichment of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea, and total ^{15}N excretion in faeces.

Ruminal degradation characteristics of CP were analyzed using the non-linear regression procedure of SAS (Version 9.1; SAS Institute, Inc. Cary, N. C.) using least squares regression (Gauss-Newton method) as described by Yu et al. (2004). Ruminal fermentation characteristics (pH, NH_3-N, osmolality and VFA concentration) were analyzed using the Proc Mixed repeated measures procedure of SAS. All other data were analyzed as a 4 × 4 Latin square design with a factorial arrangement of dietary treatments using the Proc Mixed procedure of SAS. The following model was used: $Y_{ijkl} = \mu + H_i + P_j + CP_k + RDP_l + (CP \times RDP)_{kl} + \varepsilon_{ijkl}$, where Y_{ijkl} = dependent variable, μ = overall mean, H_i = random effect of heifer i, P_j = fixed effect of period j,

CP_k = fixed effect of dietary CP k, RDP_l = fixed effect of dietary RDP_l, $(CP \times RDP)_{kl}$ = fixed effect of the interaction between CP_k and RDP_l, and ε_{ijkl} = random residual error. When the RDS × RDP interaction was significant, means were compared using the LSD test. Treatment differences were considered significant when $P \leq 0.05$ and tendencies were discussed when $0.05 < P \leq 0.10$.

3.4 Results

3.4.1 Dietary Characteristics

Chemical composition of ingredients is presented in Table 3.1. Ingredient and chemical composition of experimental diets are presented in Table 3.2. Chemical analysis of TMR indicated that CP levels were 10.8 and 14.0%. In situ ruminal incubation of dietary ingredients showed that dietary RDP levels were 76.0 and 73.6% of CP for the low and high RDP treatments, respectively (Table 3.3).

3.4.2 Ruminal Fermentation Characteristics

Ruminal pH averaged 6.1 ± 0.15 and was unaffected ($P > 0.05$) by dietary treatment (Table 3.4). Ruminal NH_3-N concentration was lower ($P < 0.05$) in heifers fed the low as compared with those fed the high CP diet (5.06 vs. 8.34 mg dL^{-1}), but was unaffected ($P = 0.60$) by RDP level. Ruminal osmolality was higher ($P = 0.03$) for heifers on the low as compared with those on the high CP diet (235.6 vs. 209.5 mOsm L^{-1}), but was unaffected ($P = 0.21$) by RDP level. Total VFA concentration, acetate:propionate ratio, and concentration of individual VFA were unaffected ($P > 0.05$) by dietary treatment, except for ruminal butyrate concentration. On

the high CP diet, ruminal butyrate concentration was higher with the high RDP level, whereas the opposite was observed on the low CP diet (interaction, $P = 0.02$).

3.4.3 Nutrient Intake and Total-tract Nutrient Digestibility

Nutrient intake and total-tract apparent digestibility of DM, OM, NDF, and ADF were unaffected ($P > 0.05$) by dietary treatment (Table 3.5).

3.4.4 Nitrogen Balance

Nitrogen intake of heifers fed the low CP diet was lower ($P < 0.01$) compared with those fed the high CP diet (134.1 vs. 168.3 g N d^{-1}; Table 3.6). Feeding the high CP diet increased ($P < 0.01$) urinary N excretion by 30% when compared with feeding the low CP diet (71.2 vs. 49.9 g N d^{-1}). However, faecal N excretion was not affected ($P > 0.05$) by dietary treatment. Nitrogen retained was greater ($P = 0.05$) on the high as compared with the low CP diet (67.7 vs. 56.5 g N d^{-1}); however, N retained, as a proportion of N intake, was unaffected ($P > 0.05$) by dietary CP level. Nitrogen loss in faeces, as a proportion of N intake, was unaffected ($P > 0.05$) by dietary treatment. Nitrogen loss in urine, as a proportion of N intake, was greater ($P = 0.04$) on the high as compared with the low CP diet (42.0 vs. 37.3 % of N intake). Nitrogen digestibility was not affected ($P > 0.05$) by dietary treatment. Plasma urea-N (PUN) concentration was greater ($P < 0.01$) on the high as compared with the low CP diet (12.9 vs. 9.6 mg dL^{-1}). Dietary RDP level did not affect ($P > 0.05$) N intake, faecal N output, urinary N output, N retention, N digestibility or PUN concentration.

Table 3.1 Chemical composition of dietary ingredients

%, DM basis	Barley	Oat hulls	Canola meal	Heated canola meal
Dry Matter (DM)	86.9	89.9	87.5	95.7
Crude Protein	10.8	3.9	43.0	41.5

Table 3.2 Ingredients and chemical composition of diets fed to beef heifers

	Low CP[z]		High CP[z]	
Item	Low[y] RDP	High[y] RDP	Low[y] RDP	High[y] RDP
Total mixed ration, % DM basis				
Barley grain	54.6	54.6	47.7	47.7
Oat hulls	35.4	35.4	32.4	32.4
Canola meal	-	4.9	7.9	14.8
Heated canola meal	4.9	-	6.9	-
Supplement	5.1	5.1	5.1	5.1
Supplement, % DM basis				
Barley grain	54.6	54.6	54.6	54.6
Limestone	20.8	20.8	20.8	20.8
Rumensin premix[x]	7.2	7.2	7.2	7.2
Trace mineral salt[w]	7.7	7.7	7.7	7.7
LS 106[v]	9.7	9.7	9.7	9.7
Chemical composition of total mixed ration				
Dry matter (DM), %	89.9	88.7	89.8	89.7
Organic matter, % DM	95.6	95.3	95.1	95.1
Crude protein (CP), % DM	10.8	10.8	13.9	14.1
Crude fat, % DM	2.1	2.1	2.5	2.3
Acid detergent fiber, % DM	17.9	16.8	18.4	18.3
Neutral detergent fiber, % DM	38.3	38.2	37.8	37.9
Neutral detergent insoluble nitrogen, % of total N	2.7	2.8	2.6	2.3
Acid detergent insoluble nitrogen, % of total N	1.7	1.7	2.3	2.2
Ruminally-degradable protein[u], % of CP	74.4	77.1	72.3	74.9

[z]CP = crude protein of 10.8 and 14.0% for the low and high CP treatments, respectively as determined by chemical analysis of diets

[y]RDP = ruminally-degradable protein of 73.4 and 76.0% (of CP) for the low and high RDP treatments, respectively as estimated by in situ incubation of dietary individual dietary ingredients

[x]Rumensin premix: 3% monensin sodium or 30,000 mg kg^{-1} monensin sodium.

[w]TM Salt: 95% salt,12,000 ppm zinc, 10,000 ppm manganese, 4,000 ppm copper, 400 ppm iodine, 60 ppm cobalt, 30 ppm added selenium.

[v]LS 106: 440,500 IU vitamin A, and 88,000 IU vitamin D_3 kg^{-1}.

[u]Ruminal degradation of CP was estimated by in situ incubation of individual dietary ingredients

Table 3.3 In situ ruminal degradation kinetics of crude protein (CP) in dietary ingredients

	Dietary Ingredients			
Items	Canola Meal	Heated Canola Meal	Oat Hulls	Barley
S^z (%)	22.19	16.04	49.85	31.88
D^y (%)	67.29	67.33	18.37	59.41
U^x (%)	10.52	16.63	31.78	8.71
Kd^w (% h^{-1})	15.79	10.42	24.69	30.84
%BCP ^{v} (%RUP)	29.05	41.23	35.37	18.39
%EDCP^u (%RDP)	70.95	58.77	64.63	81.61

^{z}S = rapidly-degradable fraction (soluble; %)
^{y}D = slowly-degradable fraction (potential; %)
^{x}U = undegradable fraction (%)
^{w}Kd = degradation rate of the D fraction (%h^{-1})
^{v}%BCP = percent ruminally-undegradable crude protein
^{u}%EDCP = percent effective (ruminally) degradable crude protein (calculated assuming a rumen outflow rate of 6% h^{-1})

Table 3.4 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on rumen fermentation characteristics in beef heifers

	Low CP[z]		High CP[z]			*P* values		
Item	Low[y] RDP	High[y] RDP	Low[y] RDP	High[y] RDP	SEM[x]	CP	RDP	CP × RDP
Ruminal pH	6.00	6.25	6.08	6.05	0.153	0.68	0.47	0.38
Ammonia-N, mg dL^{-1}	5.06	5.06	8.82	7.85	0.894	<0.01	0.60	0.60
Osmolality, mOsm L^{-1}	242.2	229.0	215.9	203.0	10.56	0.03	0.21	0.99
Volatile fatty acids, m*M*								
Acetate (A)	55.6	59.7	61.5	62.1	5.52	0.47	0.68	0.76
Propionate (P)	48.5	35.3	38.3	36.6	5.55	0.44	0.21	0.33
Butyrate	9.26	7.59	6.88	9.67	1.427	0.86	0.51	0.02
Isobutyrate	0.22	0.24	0.23	0.24	0.029	0.93	0.78	0.82
Valerate	9.81	8.57	9.04	9.07	0.922	0.88	0.48	0.46
Isovalerate	1.00	0.88	0.92	0.93	0.094	0.87	0.49	0.46
Total VFA	123.5	112.3	116.9	118.6	4.75	0.97	0.24	0.11
A:P ratio	1.34	2.29	1.89	1.95	0.471	0.83	0.31	0.37

[z]CP = crude protein of 10.8 and 14.0% for the low and high CP treatments, respectively as determined by chemical analysis of diets

[y]RDP = ruminally-degradable protein of 73.4 and 76.0% (of CP) for the low and high RDP treatments, respectively as estimated by in situ incubation of dietary individual dietary ingredients

[x]SEM = Pooled standard error of the mean

Table 3.5 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on intake and total-tract nutrient apparent digestibility in beef heifers

	Low CP[z]		High CP[z]			*P* values		
Item	Low[y] RDP	High[y] RDP	Low[y] RDP	High[y] RDP	SEM[x]	CP	RDP	CP × RDP
Dry matter (DM)								
Intake, kg d^{-1}	7.75	7.52	7.63	7.63	0.466	0.98	0.70	0.70
Total-tract digestibility, %	71.9	73.9	74.3	72.7	1.16	0.61	0.83	0.16
Organic matter (OM)								
Intake, kg d^{-1}	7.41	7.19	7.25	7.25	0.443	0.87	0.70	0.69
Total-tract digestibility, %	73.1	75.0	75.3	74.0	1.19	0.57	0.74	0.17
Neutral detergent fiber (NDF)								
Intake, kg d^{-1}	2.98	2.87	2.87	2.88	0.159	0.68	0.73	0.61
Total-tract digestibility, %	45.5	48.4	48.4	46.0	3.79	0.92	0.94	0.36
Acid detergent fiber (ADF)								
Intake, kg d^{-1}	1.39	1.26	1.39	1.40	0.085	0.37	0.41	0.34
Total-tract digestibility, %	41.0	41.9	45.3	42.3	3.93	0.53	0.74	0.46

[z]CP = crude protein of 10.8 and 14.0% for the low and high CP treatments, respectively as determined by chemical analysis of diets

[y]RDP = ruminally-degradable protein of 73.4 and 76.0% (of CP) for the low and high RDP treatments, respectively as estimated by in situ incubation of dietary individual dietary ingredients

[x]SEM = Pooled standard error of the mean

3.4.5 Microbial Protein Production

Microbial N supply averaged 61.0 g N d^{-1} and the efficiency of microbial N production averaged 11.6 g N kg^{-1} OMTDR, but neither were affected ($P > 0.05$; Table 3.7) by treatment when estimated by urinary excretion of purine derivatives.

3.4.6 Urea-N Kinetics

Endogenous production of urea (UER) was higher ($P = 0.03$; Table 3.8) in heifers fed the high as compared with the low CP diet (166.3 vs. 133.3 g d^{-1}), but was unaffected ($P = 0.84$) by dietary RDP level. The amount of endogenous urea production partitioned to the GIT (GER; $P \geq 0.18$) and urea-N utilized for anabolic purposes (UUA; $P \geq 0.72$) were unaffected by dietary treatment. Urea-N returned to the ornithine cycle (ROC) tended ($P = 0.07$) to increase as dietary CP level increased. Urinary urea-N excretion (UUE; $P = 0.04$) increased as dietary CP level increased. On the low CP diet, UUE tended to increase with the high RDP level, whereas the opposite was observed on the high CP diet (tendency for an interaction, $P = 0.06$). Urea-N transferred to the faeces (UFE; $P = 0.06$) tended to decrease as dietary CP level increased. Fractional transfers of urea-N were largely unaffected by dietary treatment; however, the proportion of gastrointestinal entry rate of urea-N (GER) that was voided in the faeces (GER to feces; $P = 0.02$) decreased as dietary CP level increased.

Table 3.6 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on nitrogen (N) intake, retention, digestibility and plasma urea-N concentrations in beef heifers

	Low CP[z]		High CP[z]			*P* values		
Item	Low[y] RDP	High[y] RDP	Low[y] RDP	High[y] RDP	SEM[x]	CP	RDP	CP × RDP
N intake, g d^{-1}	138.5	129.7	165.2	171.4	11.32	<0.01	0.86	0.34
Faecal N, g d^{-1}	28.4	26.7	28.8	30.6	2.01	0.34	0.98	0.43
Urinary N, g d^{-1}	50.7	49.0	72.9	69.5	7.08	<0.01	0.58	0.86
UUN[w]:urine-N ratio	0.69	0.82	0.88	0.73	0.107	0.69	0.92	0.25
N retained, g d^{-1}	59.1	53.8	63.7	71.6	5.64	0.05	0.79	0.21
N retained, % of N intake	42.4	41.4	38.5	42.2	2.00	0.40	0.50	0.24
N in faeces, % of N intake	20.9	20.5	17.5	18.2	1.63	0.13	0.91	0.77
N in urine, % of N intake	36.7	37.9	44.1	39.8	2.47	0.04	0.42	0.18
N digestibility, %	79.1	79.5	82.5	81.8	1.63	0.13	0.91	0.77
Plasma Urea-N, mg dL^{-1}	10.3	8.9	13.6	12.3	0.87	<0.01	0.15	0.90

[z]CP = crude protein of 10.8 and 14.0% for the low and high CP treatments, respectively as determined by chemical analysis of diets

[y]RDP = ruminally-degradable protein of 73.4 and 76.0% (of CP) for the low and high RDP treatments, respectively as estimated by in situ incubation of dietary individual dietary ingredients

[x]SEM = Pooled standard error of the mean

[w]UUN = urinary urea-nitrogen

Table 3.7 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on microbial nitrogen (N) supply as measured by purine derivatives (PD) in beef heifers

	Low CP[z]		High CP[z]			*P* values		
Item	Low[y] RDP	High[y] RDP	Low[y] RDP	High[y] RDP	SEM[x]	CP	RDP	CP × RDP
Organic matter intake, kg/d	7.4	7.2	7.3	7.3	0.44	0.87	0.70	0.69
Organic matter digestibility, %	73.1	75.0	75.3	74.0	1.19	0.57	0.74	0.17
OMTDR[w], kg/d	5.6	5.3	5.1	5.3	0.43	0.38	0.99	0.34
Urinary excretion								
Total output, kg/d	4.7	4.5	6.7	5.9	0.65	<0.01	0.09	0.28
Allantoin, mmol/d	104.7	114.3	128.7	124.4	11.55	0.11	0.76	0.51
Uric acid, mmol/d	10.9	8.2	9.4	12.7	2.51	0.34	0.86	0.14
Total PD, mmol/d	115.9	122.4	138.0	136.7	13.49	0.10	0.77	0.72
Microbial N supply[v]								
g microbial N/d	52.0	56.7	68.2	67.2	9.53	0.10	0.78	0.72
g microbial N/kg OMTDR[w]	9.8	10.5	14.1	12.0	2.46	0.21	0.71	0.57

[z]CP = crude protein of 10.8 and 14.0% for the low and high CP treatments, respectively as determined by chemical analysis of diets

[y]RDP = ruminally-degradable protein of 73.4 and 76.0% (of CP) for the low and high RDP treatments, respectively as estimated by in situ incubation of dietary individual dietary ingredients

[x]SEM = Pooled standard error of the mean

[w]Microbial N supply per kg of organic matter (OM) truly digested in the rumen (OMTDR)

[v]Microbial N supply was calculated according to the methods of Chen and Gomes (1992)

Table 3.8 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on urea-nitrogen (N) recycling kinetics, as measured by continuous jugular infusions of [$^{15}N^{15}N$]-urea in beef heifers

	Low CP[z]		High CP[z]			*P* values		
Item	Low[y] RDP	High[y] RDP	Low[y] RDP	High[y] RDP	SEM[x]	CP	RDP	CP × RDP
Urea-N fluxes, g d^{-1}								
UER[w]	135.0	131.7	166.9	165.7	10.81	0.03	0.84	0.92
GER[v]	98.4	93.3	105.6	121.1	11.35	0.18	0.67	0.40
UUA[u]	25.4	20.9	17.2	35.1	7.71	0.72	0.43	0.21
ROC[t]	70.3	69.8	86.2	84.0	6.47	0.07	0.84	0.90
UUE[s]	36.6	38.4	61.3	44.6	5.06	0.04	0.35	0.06
UFE[r]	2.69	2.58	2.13	2.05	0.220	0.06	0.68	0.95
Fractional transfers								
UER to urine	0.27	0.31	0.37	0.28	0.035	0.38	0.50	0.11
UER to GIT[q]	0.73	0.69	0.63	0.72	0.035	0.38	0.50	0.11
GER to ROC	0.71	0.75	0.82	0.70	0.055	0.64	0.49	0.18
GER to faeces	0.03	0.03	0.02	0.02	0.002	0.02	0.93	0.74
GER to UUA	0.26	0.22	0.16	0.29	0.057	0.76	0.50	0.19

[z]CP = crude protein of 10.8 and 14.0% for the low and high CP treatments, respectively as determined by chemical analysis of diets

[y]RDP = ruminally-degradable protein of 73.4 and 76.0% (of CP) for the low and high RDP treatments, respectively as estimated by in situ incubation of dietary individual dietary ingredients

[x]SEM = Pooled standard error of the mean

[w]UER = Urea-N entry rate.

[v]GER = Gastro-intestinal entry rate.

[u]UUA = Urea-N utilized for anabolism.

[t]ROC = Return to ornithine cycle.

[s]UUE = Urinary urea-N excretion.

[r]UFE = Urea-N loss to faeces.

[q]GIT = Gastro-intestinal tract.

3.5 Discussion

Beef cattle retain approximately 25% of their dietary N intake with the remaining N excreted in the urine (39%) and faeces (29%) (Biermen 1999; Gaylean 1996). Furthermore, between 50 and 90% of urinary N is excreted as urea-N (Reynal and Broderick 2005) which is highly volatile in the environment, leading to pollution of the atmosphere as NH_3 and N_2O (Baggs and Philippot 2010), and soil and ground water as nitrate (Socolow 1999; Cowling and Galloway 2002). Public concern over the environmental impact of intensive livestock operations is increasing (Janzen 2011), making it important to address the efficiency of nutrient utilization. This study attempted to manipulate the amount and form of dietary protein in an effort to minimize excretion of N into the environment and improve the overall efficiency of N use in beef heifers.

In the present study, endogenous production of urea (UER) was higher (P = 0.03) in heifers fed the high CP diet (14.0% CP) as compared with those fed the low CP diet (10.8% CP). Previous research has clearly demonstrated that UER increases as dietary CP intake increases (Archibeque et al. 2001; Marini and Van Amburgh 2003; Marini et al. 2004; Brake et al. 2010). Lobley et al. (2000) observed an increase UER in sheep as dietary N intake increased. Furthermore, UER tended to be greater in steers fed a corn-based diet containing DDGS as compared to a control diet (14.9 vs. 10.2% CP, respectively) (Brake et al. 2010). This is explained by the fact that dietary protein is degraded to NH_3 by ruminal bacteria (Bach et al. 2005) and that NH_3 produced in excess of bacterial requirements for protein synthesis is absorbed systemically and transported to the liver and used for urea production via the ornithine cycle (Stewart and Smith 2005). This urea can either be returned to the GIT or excreted in the urine (Harmeyer and Martens 1980; Lapierre and Lobley 2001). In the current study both GER

and UUA were unaffected by dietary treatment ($P > 0.05$). However, GER was numerically greater in heifers fed the high CP diet compared with the low CP diet. Previous research which showed that GER increases as dietary CP decreases (Marini and Van Amburgh 2003), attributed this response to a lower ruminal NH_3-N concentration (Kennedy and Milligan 1980). Bacterial urease facilitates the transfer of urea-N across the ruminal epithelium (Rémond et al. 1996) but the activity of bacterial urease decreases as ruminal NH_3-N concentration increases (Marini et al. 2004). In the present study ruminal NH_3-N concentrations increased ($P < 0.01$) with an increase in dietary CP concentration. The increase in ruminal NH_3-N concentration may therefore have reduced GER on the high CP diets due to a reduction in ruminal epithelium's permeability to urea-N via a decrease in bacterial urease activity.

Several reports in the literature have demonstrated a direct association between dietary CP concentration and ruminal NH_3-N concentration (Cunningham et al. 1996; Kebreab et al. 2002; Reynal and Broderick 2005). Kiran and Mutsvangwa (2010) found that reducing dietary CP concentration from 15% to 10% (DM basis) reduced ruminal NH_3-N concentration by approximately 42% in sheep, while reducing CP content from 18.6% to 17.5 % in lactating dairy diets reduced ruminal NH_3-N concentration from 12.0 to 7.2 mg dL^{-1} (Reynal and Broderick 2005). Ammonia is required for microbial protein production (Wallace 1997) and reducing ruminal NH_3-N concentration below a critical threshold could potentially impede microbial protein production (Balcells et al. 1993). Previous in vitro studies have concluded that thiscritical threshold is 5 mg of NH_3 dL^{-1} of ruminal fluid (Satter and Slyter 1974; Russell and Strobel 1987). However, in vivo estimates have shown that optimal ruminal NH_3-N concentration for microbial protein synthesis depends on the fermentability of the diet. Several authors have concluded that as the fermentability of the diet increases so does the requirement for ruminal

NH_3-N to maximize microbial protein production (Erdman et al. 1986; Odle and Schaefer 1987). For example, Odle and Schaefer (1987) demonstrated that barley fed steers required a minimum ruminal NH_3-N concentration of 12.5 mg dL^{-1} to maximize ruminal nutrient whereas corn fed steers only required 6.1 mg dL^{-1}. In the present study, heifers were fed a diet consisting of approximately 34% (DM basis) oat hulls, which have a relatively high lignin content and low ruminal degradability (Thompson et al. 2000; Thompson et al. 2002). Although ruminal NH_3-N concentrations on the low CP diet may be considered marginal (5.1 mg dL^{-1}), it still met the critical threshold proposed by others (Satter and Slyter 1974; Russell and Strobel 1987).

Although N intake differed among diets ($P < 0.01$), microbial protein flow to the duodenum was not significantly affected by dietary treatment. Dietary protein is extensively degraded in the rumen to peptides, AA, and NH_3 which are subsequently utilized for microbial growth (Bach et al. 2005). Therefore, increasing protein intake often results in increases in microbial protein synthesis (Wickersham et al. 2008a; Kiran and Mutsvangwa 2010). These results indicate that feeding growing beef heifers a low CP diet (10.8% CP) will reduce urinary N excretion without significantly impacting microbial protein flow to the small intestine, indicating a greater efficiency of N utilization by heifers fed the low CP diet as compared with the high CP diet. One mechanism improving the efficiency of N utilization could be the increased capture of recycled urea-N by ruminal microbes as has been reported in the literature. Brake et al. (2010) found that the proportion of recycled urea-N in microbial N was greater in steers fed a control diet (10.2% CP) than steers supplemented with either urea (13.3 % CP) or DDGS (14.9% CP).

In the present study, in situ estimates of dietary RDP level showed a lower than expected spread between the low and high RDP treatments. Ruminally-degradable protein values were

manipulated by the use of regular and heated canola meal. Typically, regular canola meal has a RUP value of 30% of CP (NRC 1996). Heat treatment of canola meal has been shown to increase the RUP fraction of the meal (McKinnon et al. 1995) to values approaching 60% of CP (NRC 1996). The magnitude of the increase in RUP is influenced by the temperature and duration of heating (McKinnon et al. 1991). In this study, the heated canola meal was obtained from a commercial source with a stated RUP content of 70% of CP (Canbra Foods Ltd 2007). In situ incubation of the regular and heated canola meal indicated RUP values of 29.0 and 41.5% of CP, respectively. This difference between actual (41.5%) and expected (70%) RUP of the heated canola meal explains the lower than expected RUP level of the total mixed ration. The fact that differences between dietary RDP levels were lower than expected could account for the reduced effect of dietary RDP level on urea kinetics in this study as compared to previous studies. For example, when supplementing forage-fed steers with different levels of casein, to manipulate RDP level, Wickersham et al. (2008) found that GER increased as the level of casein supplementation increased.

Ruminal pH is influenced by total VFA concentration (Beauchemin et al. 2003). It has been shown that as the amount of readily fermentable carbohydrate increases, ruminal VFA concentration increases and, as a consequence, ruminal pH decreases (Nocek 1997; Bach et al. 2005). In this study, heifers were fed isoenergetic diets containing a slowly fermentable forage source (i.e., oat hulls). This explains the limited effect of diet on VFA concentration and ruminal pH ($P > 0.05$). Normal ruminal osmolality levels are between 240 and 300 mOsm L^{-1} (Owens et al. 1998). Animals in the present study had lower than normal levels of ruminal osmolality. Ruminal osmolality is a measure of dissolved solutes (i.e. minerals, VFA, and lactate; Owens et al. 1998) and as such, osmolality increases with increasing concentrate in the diet (Brown et al.

2000). The low osmolality values observed likely reflect the poor ruminal digestibility of oat hulls (Thompson et al. 2000).

A reduction in dietary CP from 14.0 to 10.8% did not impact nutrient intake or total-tract apparent digestibility of DM, OM, N, NDF, and ADF. Low CP diets (12%) have been reported to negatively influence feed digestibility in first lactation Holstein cows (MacLeod et al. 1982) as well as reduce microbial growth and fermentation activity (Martin-Orue et al. 2000) leading to a reduction in ruminal nutrient disappearance (Klevesahl et al. 2003). These results along with ruminal NH_3-N levels indicate that lowering dietary CP level to 10.8% did not negatively impact overall digestibility of dietary nutrients and that ruminal NH_3-N levels were at or above the critical threshold reported by Satter and Slyter (1974) and sufficient to support microbial fermentation activity in beef heifers.

Feeding the high CP (14.0%) diet increased ($P < 0.01$) urinary N excretion by 30% compared to the low CP (10.8%) diet, a finding in agreement with several other studies (Archibeque et al. 2001; Marini and Van Amburgh 2003; Marini et al. 2004; Kiran and Mutsvangwa 2010). This shows that reducing dietary N intake is an effective nutritional strategy for reducing the excretion of urinary N into the environment. Urinary N is mainly excreted as urea (Broderick 2003), which can be rapidly converted to NH_3 by anaerobic and aerobic bacteria present in the environment (McGinn et al. 2002; Hristov et al. 2011). This is evident from the work of McGinn et al. (2002) who showed a positive relationship between dietary CP level and the concentration of NH_3-N in beef cattle manure. In this study, between 36.6 and 61.3% of urinary N was excreted as urinary urea-N by heifers, with lower values attributed to the low CP diet. These results further support the concept that the potential impact of cattle on the environment can be reduced by lowering the CP content of the diet (McGinn et al. 2002;

Reynolds and Kristensen 2008; Hristov et al. 2011). Faecal N excretion was not affected by increasing the level of dietary CP. Hristov and Jouany (2005) reported that faecal N output is less affected by dietary N intake than urinary N output, and that excess dietary N is more readily partitioned to the urine. This is in agreement with previously reported data (Siddons et al. 1985; Marini and Van Amburgh 2003). However, the proportion of GER excreted in the faeces was lower ($P = 0.02$) for heifers fed the low CP diets as compared with the high CP diets. Nitrogen digestibility was not affected ($P > 0.05$) by dietary treatment. It has previously been shown that reducing dietary CP concentration leads to a decrease in fiber digestibility (Willms et al. 1991). The fact that this did not occur is further evidence that in the present study the low dietary CP diet did not significantly restrict ruminal microbial fermentation.

Nitrogen retention was greater on the high CP diet as compared with the low CP diet as has been previously reported (Willms et al. 1991; Wright et al. 1998). However, N retained as a proportion of N intake was not affected ($P > 0.05$) by an increase in N intake. This result has been previously observed in sheep (Kiran and Mutsvangwa 2007; Kiran and Mutsvangwa 2010). This is also in agreement with findings of Archibeque et al. (2001), where efficiency of N use was not affected by N intake in beef heifers. Failure to see an increase in N retained, as a proportion of N intake, is explained by the observation that N supplied in excess of requirements is excreted as urea-N in the urine and this represents a waste of feed N (Lapierre and Lobley 2001). Nitrogen loss in urine, as a proportion of N intake, was greater ($P = 0.04$) on the high CP diet as compared with the low CP diet. Whereas, N loss in faeces, as a proportion of N intake, was not affected ($P > 0.05$) by dietary treatment and further illustrates the fact that excess dietary N is more readily partitioned to the urine than the faeces (Hristov and Jouany 2005). Plasma urea-N concentration was greater ($P < 0.01$) in heifers fed the high CP diet as compared with

those fed the low CP diet. This would be expected due to the greater N intakes and subsequent higher ruminal NH_3-N concentration leading to greater rates of ruminal NH_3-N absorption. In agreement with this result, Marini et al. (2004) reported a linear increase in PUN concentrations with increasing N intake in ewe lambs.

In conclusion, lowering dietary CP level reduced urinary N excretion and did not affect nutrient digestibility or microbial protein supply to the duodenum. Results also indicate that GER was similar across diets, and that most of the urea which entered the GIT was returned to the ornithine cycle. The implications of these findings are that lowering dietary CP to 10.8% can reduce the excretion of N into the environment and improve the efficiency of N utilization with no significant change in microbial protein production.

4 EFFECTS OF DIETARY RUMINALLY-DEGRADABLE STARCH AND RUMINALLY-DEGRADABLE PROTEIN LEVELS ON UREA RECYCLING, MICROBIAL PROTEIN PRODUCTION, NITROGEN BALANCE, AND DUODENAL NUTRIENT FLOW IN BEEF HEIFERS FED LOW CRUDE PROTEIN DIETS[1]

4.1 Abstract

The objective of this study was to determine the effects of ruminally-degradable starch (RDS; 28.6 and 69.2% of total starch) and ruminally-degradable protein (RDP; 48.0 and 55.0% of crude protein [CP]) content on urea recycling, nitrogen (N) balance, duodenal nutrient flow, and microbial protein production in beef heifers fed low CP (10%) diets. Four ruminally- and duodenally-cannulated beef heifers (723 ± 57 kg BW) were used in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of dietary treatments with 23-d periods. Jugular infusions of [$^{15}N^{15}N$]-urea (220 mg d^{-1}; 98+ atom percent) were conducted for 4 d (d 18-22) to estimate urea kinetics, with total collection of faeces and urine. Proportions of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea in urinary urea, and ^{15}N enrichment in faeces were used to calculate urea kinetics. Ruminal microbial N production was estimated using ^{15}N as a marker. Ruminal ammonia-N concentration was greater (P = 0.01) in heifers fed high RDP as compared with those fed low RDP, and it was also greater (P = 0.01) in heifers fed low RDS as compared with those fed high RDS. Microbial N flow to the duodenum increased as RDP level increased on the high RDS diet, but was not affected by RDP level on the low RDS diet (interaction; P = 0.04). Urea-N entry rate and urea-N transfer to the gastro-intestinal tract were similar (P > 0.05) across diets. The amount of recycled

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urea-N incorporated into microbial N increased as RDP level increased on the high RDS diet, but the opposite was observed on the low RDS diet (interaction; $P = 0.008$). These results indicate that at a low CP level (10%), increasing both RDS and RDP levels can increase microbial N flow to the duodenum and improve the efficiency of use of recycled urea-N for microbial N synthesis.

4.2 Introduction

There has been increasing concern over the impact of animal agriculture on the environment (Socolow 1999; Food and Agriculture Organization of the United Nations 2006), in particular, the overfeeding of nutrients such as nitrogen (N) (Cowling and Galloway 2002). One strategy to reduce N excretion in livestock waste is to reduce dietary crude protein (CP) concentration (Hristov et al 2011). However, a low dietary CP content will restrict microbial protein synthesis due to a shortage of ruminally degradable protein (RDP). Reduced microbial growth will, in turn, lead to a reduction in ruminal fermentation activity and, thus, a reduction in the digestibility of nutrients, thereby reducing animal performance (Clark and Overton 1995).

In ruminants, there is a constant recycling of urea between the liver and the gastro-intestinal tract (GIT), a mechanism that conserves N (Stewart and Smith 2005). The recycling of urea can contribute to the ruminal ammonia (NH_3) pool for microbial production and improve efficiency of conversion of feed N to animal products (Lapierre and Lobley 2001). It has been shown that urea recycling to the GIT is enhanced on low RDP diets (Marini and Van Amburgh 2003) and is associated with decreased ruminal ammonia-N (NH_3-N) concentration (Kennedy and Milligan 1980). It has also been shown that more severe processing of cereal grains can shift the site of starch digestion from the small intestine to the rumen (Huntington 1997; Theurer et al. 1999). Greater ruminally-degradable starch (RDS) availability also increases the proportion of

urea recycled to the GIT due to increased microbial utilization of ruminal NH_3 (Kennedy and Milligan 1978; Kennedy and Milligan 1980; Huntington 1989). However, there is limited information on how simultaneous changes in dietary content of RDP and RDS influence urea kinetics and microbial production in heifers fed low CP diets.

The hypothesis of this study was that with beef heifers fed low CP diets, urea recycling to the GIT will be enhanced at a low dietary RDP level and that a greater proportion of this recycled urea will be captured for microbial protein production when the dietary level of RDS is increased. The objective was to determine the effects of feeding diets formulated to contain two levels of RDS (30 and 70% of total starch) and two levels of RDP (48% vs. 64% of CP) on urea recycling to the GIT, N balance, duodenal nutrient flow, and microbial protein production in beef heifers fed low CP diets.

4.3 Materials and Methods

Heifers used in this study were cared for in accordance with the guidelines of the Canadian Council of Animal Care (1993) and their use was approved by the University of Saskatchewan Animal Care Committee (UCACS Protocol No. 20040048).

4.3.1 Animals and Experimental Design

Four Speckle Park beef heifers (723 ± 57 kg BW) surgically fitted with ruminal (10 cm diameter opening; Bar Diamond, Parma, ID) and duodenal T-type (2.5 cm internal diameter; Lethbridge Research Centre, Lethbridge, AB) cannulas were used in a 4 × 4 Latin square design experiment with a 2 × 2 factorial arrangement of treatments and 23-d experimental periods.

Duodenal cannulas were placed proximal to the common bile and pancreatic ducts, approximately 10 cm distal to the pylorus. Each experimental period consisted of 18 d of dietary adaptation and 5 d of sample collection. Heifers were housed in individual 3 m × 3 m pens on rubber mats in the Livestock Research Building of the Department of Animal and Poultry Science (University of Saskatchewan). Dietary treatments consisted of two levels of RDS (28.6 and 69.2% of total starch) and (48.0 and 55.0% of CP). Dietary levels of RDS were manipulated by feeding either whole-shelled corn or steam-rolled corn, obtained from the same lot. Dietary RDP levels were manipulated by feeding either canola meal or fishmeal as the protein supplement. All dietary ingredients except for the corn grain were pelleted. The steam-rolled corn was processed at a rolling temperature of 96 to 99^{o}C, with an average retention time in the steam chest of 15 to 20 min and an average gap between rolls of 4 mm. All diets were formulated to contain 10% CP (dry matter [DM] basis). Experimental diets were fed twice daily at 0800 and 1600 h and heifers had free access to water. Ingredient and chemical composition of experimental diets are presented in Table 4.1. Chemical composition of ingredients is presented in Table 4.2.

4.3.2 Sample Collection

During the 5-d collection period (d 18 to d 23), individual heifer feed intake was recorded daily. Also, samples of total mixed ration (TMR) and orts were collected daily, stored at -20ºC and then composited by treatment for each experimental period.

On d 17 of each experimental period, heifers were fitted with temporary vinyl catheters (0.86 mm i.d. × 1.32 mm o.d.; Scientific Commodities Inc., Lake Havasu City, AZ) in both the right and left jugular veins to allow for simultaneous blood sampling and isotope infusion. Total

N balance (d 18 to d 23) and urea-N transfer to the GIT (d 18 to d 22) were determined using the procedures of Lobley et al. (2000), except that urine was collected using bladder catheters. During total collection of urine and faeces, heifers were restrained within the pens. On d 17, indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) were inserted into heifers as described by Crutchfield (1968). Just before 0800 h on d 18, background samples of urine, faeces, and ruminal and duodenal contents were collected to measure ^{15}N natural abundance (NA), and the bladder catheters were connected to urine collection tubing. Starting at 0800 h on d 18, double-labeled urea ([$^{15}N^{15}N$]-urea, 98+ atom percent; Cambridge Isotope Laboratories, MA, USA) dissolved in 2 L of sterile saline solution was continuously infused into the jugular vein at a rate of 220 mg d^{-1} using a peristaltic pump (Watson and Marlow, Cornwall, UK. Model: 205U) for 96 h. Urine was collected into 20 L Carboy polyethylene containers into which 80 mL of concentrated HCl (VWR Scientific, Mississauga, ON) had been added to achieve a urine pH < 3. The acidification of urine was necessary to avoid the loss of volatile NH_3-N, which would lead to the underestimation of N excretion. Total daily urine output for each heifer was weighed, mixed thoroughly and a sample (20% of total output) collected. A 50 mL sub-sample of urine was collected from the composite daily output. In addition, a 2 mL sub-sample of urine was collected daily from the composite daily output and diluted with 8 mL of distilled water. All urine samples were stored at -20^{o}C. Total faecal output was collected daily for 5 d (d 18 to d 23 of each experimental period). Faecal samples (10% of total output) were taken daily, a sub-sample was taken and the remainder composited by heifer per period and stored at -20^{o}C.

Ruminal, duodenal and blood samples were collected every 3 h for 24 h (0800 h on d 21 to 0500 h on d 22). Ruminal contents (1 L; 250 mL from the cranial ventral, caudal ventral,

central, and cranial dorsal regions) were collected, mixed thoroughly and strained through four layers of cheesecloth. Ruminal fluid pH was immediately determined on the filtrate, using a Model 265A portal pH meter (Orion Research Inc., Beverly, MA). A 5 mL aliquot of ruminal fluid was preserved with 1 mL of meta-phosphoric acid (25% wt/vol), and a second 5-mL aliquot was preserved with 1 mL of 1% sulphuric acid and stored at -20^{o}C. A third 15-mL aliquot was not acidified and was stored at -20 ^{o}C for later determination of osmolarity. The solid ruminal digesta that was retained on the cheesecloth was mixed with 400 mL of saline solution and then homogenized in a blender to dislodge particle-associated bacteria. The blended digesta was then strained through four layers of cheesecloth. The resulting fluid was mixed with the previously strained ruminal fluid and then stored on ice until further processing to isolate mixed ruminal bacteria by differential centrifugation (Reynal et al. 2005). Ruminal bacterial samples from each heifer in each period were composited and stored at -20^{o}C. Duodenal samples (500 mL) were composited for each heifer in each period and stored at -20°C. Blood samples (5 mL) were collected from a jugular catheter into tubes containing heparin (144 USP units; Becton Dickinson, Rutherford, NJ) and transferred to the laboratory on ice. Blood samples were centrifuged at 1,500 × *g* for 15 min at 4^{o}C (Beckman Instruments Inc., Model J6-MC Centrifuge, Mississauga, Ontario, Canada). The resulting plasma was stored at -20^{o}C.

Dietary contents of RDP and RDS of the TMR were determined using the in situ incubation technique (Yu et al. 2004) with two beef heifers (average BW 784 kg) that were fitted with ruminal cannula (10 cm diameter opening, Bar Diamond, Parma, ID). The animals were housed in individual 3 m × 3 m pens in the Livestock Research Building (University of Saskatchewan) and were fed a blend of all four experimental diets at 2% of body weight (DM basis) at 0800 and 1600 h daily. Briefly, samples of each of the diet pellets and the steam-rolled

corn were ground through a 3 mm screen (Christy and Norris Ltd., Chelmsford, England), and whole-shelled corn was crushed with a hammer to minimize grain shattering. The pellets and corn were then combined in the correct ingredient proportions as in the TMR before a 7 g sample was weighed into number-coded bags (10 cm × 20 cm, Nitex 03-41/31 monofilament open mesh fabric, Screentec Corp., Mississauga, ON; with a 40 μm pore size). Incubations were carried out for 72, 48, 24, 12, 8, 4, 2, and 0 h. Procedures for ruminal incubation and post-incubation processing of bags were as described by Yu et al. (2004). Two incubation runs were conducted and, within each incubation run, all diets were randomly incubated in duplicate in the two heifers.

4.3.3 Sample Analyses

After completion of the experiment, frozen TMR, orts and faecal samples were thawed at room temperature and then dried at 55^{o}C for 96 h. Dried TMR, orts, and faecal samples were ground through 1 mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Samples were then analyzed for DM by oven-drying at 135°C for 2 h [Association of Official Analytical Chemists (AOAC) 1990; method 930.15], OM by ashing at 600^{o}C for at least 8 h (AOAC 1990; method 942.05), CP using the macro-Kjeldahl procedure (AOAC 1990; method 984.13), ether extract (AOAC 1990; method 920.39), starch (AOAC 1990; method 996.11), acid detergent fiber (ADF) (AOAC 1990; method 973.18), and neutral detergent fiber (NDF; Van Soest et al. 1991). Amylase and sodium sulfite were used for NDF determination. Dried TMR were analyzed for acid detergent insoluble (ADIN) and neutral detergent insoluble (NDIN) nitrogen (Licitra et al. 1996), and acid detergent insoluble ash (ADIA; Van Soest et al. 1991). Prior to chemical analysis, the dried residues from in situ incubations were ground

through a 1 mm screen using a Retch ZM 100 grinder (F-Kurt Retsch GmbH & Co. Kg, Germany) and then analyzed for DM and starch as previously described, and CP using the Dumas method of combustion on the LECO FP-528 Nitrogen/Protein Determinator (AOAC 1990; method 992.23. Duodenal samples were freeze-dried (Virtus '72, Gardner, New York) and then ground in a Braun Aromatic coffee grinder (KSM 2, 2.5 oz). Ground duodenal samples were analyzed for OM, NDF, ADF, starch, and ADIA using the procedures described above.

Urinary urea-N was determined using the diacetyl monoxime method (Procedure No. 0580, Stanbio Laboratory, Boerne, TX) on background and daily composite 50 mL subsamples of urine prior to processing for the determination of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea enrichments. Urinary urea was then isolated by applying urine containing 1.5 mg of urea-N to a pre-packed cation exchange resin column (AG-50W- × 8 Resin, 100-200 mesh, H+ form; BioRad Laboratories, Hercules, CA) as described by Archibeque et al. (2001). Samples were then eluted with N-free water, air-dried and transferred to borosilicate glass tubes for freeze-drying and then analyzed for the proportions of [$^{15}N^{15}N$]-, [$^{14}N^{15}N$]-, and [$^{14}N^{14}N$]-urea in urinary urea by isotope ratio-mass spectrometry (^{15}N Analysis Laboratory, University of Illinois at Urbana-Champaign). The results were corrected for [$^{14}N^{15}N$]-urea produced by non-monomolecular reactions (Lobley et al. 2000). Total N in samples of daily urine output was determined using the macro-Kjeldahl procedure (AOAC 1990; method 976.05). Diluted daily (d 19 to d 22) urine samples were composited (proportionally based on daily urine output) by heifer for each period. Frozen plasma samples were thawed and composited by heifer for each experimental period. Urea-N was determined in urine and plasma using the diacetyl monoxime method.

In preparation for ^{15}N analysis, ruminal bacterial samples were freeze-dried and finely ground with a mortar and pestle. Dried duodenal and daily faecal samples were finely ground

using a ball mill. Finely ground ruminal bacterial, duodenal, and faecal samples were prepared for ^{15}N analysis as described by Brito et al. (2006). Briefly, ruminal bacterial, duodenal, and faecal samples containing approximately 100 µg of N were weighed into 5 × 9 mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK). To volatilize NH_3-N, 50 µL of 72 m*M* K_2CO_3 was then added to each tin capsule followed by incubation in a forced-air oven at 60°C for 24 h. Enrichment of ^{15}N in ruminal bacterial, duodenal, and faecal samples was then measured by combustion to N_2 gas in an elemental analyzer and continuous flow isotope ratio-mass spectrometry (Lobley et al. 2000). Dried duodenal digesta samples were analyzed for NH_3-N as described by Brito et al. (2006). Briefly, 10 mL of sodium citrate (77.5 m*M*, pH 2.2) was added to 0.5 g of duodenal digesta sample, the mixture was mixed thoroughly by vortexing, and then held at 39°C for 30 min. Samples were subsequently centrifuged at 15,000 × g for 15 min at 4°C and NH_3-N concentration was determined in the supernatant using a phenol-hypochlorite assay (Broderick and Kang 1980). Ruminal fluid samples preserved with meta-phosphoric acid were analyzed for volatile fatty acid (VFA) concentration (Erwin et al. 1961) using an Agilent 6890 Series Gas Chromatography system (Wilmington, DE). Ruminal fluid samples preserved with sulphuric acid were analyzed for NH_3-N using a phenol-hypochlorite assay (Broderick and Kang 1980). Osmolality was measured on non-acidified ruminal fluid samples using aVapro™ Vapor Pressure Osmomemter (Model 5520; Wescor Inc., Logan, Utah).

4.3.4 Calculations and Statistical Analysis

Duodenal DM flow was determined as ADIA intake ÷ concentration of ADIA in duodenal digesta. Dietary RDP and ruminally-undegradable protein (RUP) in vivo levels were calculated as %RDP on CP basis = [(CP intake, g/d – RUP Flow, g d^{-1}) ÷ (CP intake, g d^{-1})] ×

100 and %RUP on CP basis = [(duodenal CP flow, g d^{-1} – ^{15}N bacterial CP flow, g d^{-1}) ÷ (CP intake, g d^{-1})] × 100 (Reynal and Broderick 2005). Dietary RDS was calculated as %RDS on total starch basis = [(Total starch intake, kg d^{-1} – duodenal starch flow, kg d^{-1}) / Total starch intake, kg/d)] × 100. Total N retention was calculated as intake N – faecal N – urinary N. Apparent digestion of nutrients in the rumen was calculated as nutrient intake – duodenal flow of nutrient (kg d^{-1}). Duodenal bacterial flow was determined based on calculations from Reynal and Broderick (2005). The natural abundance of ^{15}N (^{15}NA) in rumen bacteria and duodenal digesta were determined from samples collected from each heifer in each period prior to the initiation of [$^{15}N^{15}N$]-urea isotope infusion. The ^{15}N enrichment was calculated for bacterial and duodenal digesta samples for each heifer in each period as ^{15}N-atom % in sample minus individual heifer ^{15}NA. Total microbial N flowing to the duodenum was calculated using ^{15}N as a microbial marker as nonammonia-nitrogen (NAN) flow to the duodenum × (duodenal ^{15}N enrichment of NAN ÷ rumen bacteria ^{15}N enrichment), expressed in g per d. The above RUP flow (assumed to comprise of dietary and endogenous NAN) was calculated as total duodenal N flow – bacterial N flow, expressed in g per d. The flow of bacterial N derived from recycled urea-N was calculated as bacterial N flow × (bacterial ^{15}N enrichment ÷ urinary ^{15}N enrichment) (Wickersham et al. 2008a). Microbial efficiency was calculated using 2 approaches: 1) based on g of N per kg of OM truly digested in the rumen (OMTDR), calculated as microbial N flow ÷ kg of OMTDR, and 2) based on g of N per kg of total digestible organic matter intake (TDOMI), calculated as microbial N flow ÷ TDOMI. Urea-N kinetics was calculated according to the model of Lobley et al. (2000), using urinary ^{15}N enrichment of [$^{15}N^{15}N$]-urea, and [$^{14}N^{15}N$]-urea, and total ^{15}N excretion in faeces.

Ruminal degradation characteristics of CP and starch were analyzed using the non-linear regression procedure of SAS (Version 9.1; SAS Institute, Inc. Cary, N. C.) using least squares regression (Gauss-Newton method) as described by Yu et al. (2004). Ruminal fermentation characteristics (pH, NH_3-N, osmolality and VFA concentration) were analyzed using the Proc Mixed repeated measures procedures of SAS. All other data were analyzed as a 4 × 4 Latin square design with a factorial arrangement of dietary treatments using the Proc Mixed procedure of SAS. The following model was used: $Y_{ijk} = \mu + H_i + P_j + RDS_k + RDP_l + (RDS \times RDP)_{kl} + \varepsilon_{ijkl}$, where Y_{ijkl} = dependent variable, μ = overall mean, H_i = random effect of heifer i, P_j = fixed effect of period j, RDS_k = fixed effect of dietary RDS k, RDP_l = fixed effect of dietary RDP_l, $(RDS \times RDP)_{kl}$ = fixed effect of the interaction between RDS_k and RDP_l, and ε_{ijkl} = random residual error. When the RDS × RDP interaction was significant, means were compared using the LSD test. Treatment differences were considered significant when $P \leq 0.05$ and tendencies were discussed when $0.05 < P \leq 0.10$.

4.4 Results

4.4.1 Dietary Characteristics

Dietary ingredients and chemical composition are presented in Table 4.1. As expected, all diets were similar (mean ± SEM) in CP (10.0 ± 0.14%), ADF (20.4 ± 0.22%) and NDF (41.8 ± 0.41%) content. In situ ruminal incubation of TMR showed that dietary RDS levels were 28.6% and 69.2% of total starch for the low and high RDS treatments, respectively, and that dietary RDP levels were 48.0% and 55.0 % of CP for the low and high RDP treatments, respectively (Table 4.3).

4.4.2 Ruminal Fermentation Characteristics

Ruminal pH was lower ($P < 0.01$) in heifers fed high RDS as compared with those fed the low RDS diets (5.93 vs. 5.76; Table 4.4). In addition, ruminal pH was lower ($P = 0.01$) in heifers fed the high RDP as compared with those fed the low RDP diets (5.79 vs. 5.90). Ruminal NH_3-N concentration was greater ($P = 0.01$) in heifers fed high RDP as compared with those fed low RDP (8.6 vs. 6.3 mg dL^{-1}). Ruminal NH_3-N concentration was greater ($P = 0.01$) in heifers fed low RDS as compared with those fed high RDS (8.5 vs. 6.3 mg dL^{-1}). Ruminal osmolality was higher ($P = 0.02$) for heifers on the high RDP diet as compared with the low RDP diet (273.9 vs. 258.6 mOsm L^{-1}). Total and individual VFA concentrations were unaffected by dietary treatment, except for ruminal valerate concentration which was greater ($P = 0.03$) in heifers fed the low RDS diet compared with those fed the high RDS diet (0.6 vs. 0.5 m*M*).

4.4.3 Nutrient Intake, Duodenal Nutrient Flow, and Ruminal and Total-tract Nutrient Digestibility

Nutrient intake and duodenal flows of DM, OM, NDF, ADF, and starch were unaffected by dietary treatment ($P > 0.05$; Table 4.5). The percentages of DM (interaction, $P = 0.05$) and OM (tendency for an interaction, $P = 0.10$) digested in the rumen decreased as RDP level increased on the high RDS diet, but no differences were observed on the low RDS diet. Ruminal digestion of NDF, when expressed in absolute amounts (interaction, $P = 0.03$) or as a percentage of NDF intake (interaction, $P = 0.02$), decreased as RDP level increased on the high RDS diet but no differences were observed on the low RDS diet. Total tract digestibility of NDF was greater ($P = 0.03$) on the low RDP diet as compared with the high RDP diet (48.6 vs. 41.4%). The amount of ADF digested in the rumen tended ($P = 0.08$) to decrease as RDP level increased on the high

Table 4.1 Ingredient and chemical composition of diets fed to beef heifers

	Low RDS[z]		High RDS[z]	
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]
Total mixed ration, % DM basis				
Oat hulls	49.6	48.9	47.0	46.1
Steam-rolled corn	-	-	42.4	42.6
Whole-shelled corn	40.9	42.9	-	-
Canola meal	-	3.0	-	6.0
Fishmeal	4.4	-	5.5	-
Supplement	5.1	5.2	5.1	5.3
Supplement, % DM basis				
Barley grain	51.9	38.3	56.6	45.3
Limestone	18.6	18.3	18.7	20.6
Rumensin premix[x]	7.2	7.1	7.3	7.2
Urea	4.9	19.2	-	9.7
Trace mineral salt[w]	7.7	7.6	7.7	7.6
LS 106[v]	9.7	9.5	9.7	9.6
Chemical composition of total mixed ration				
Dry matter (DM), %	91.4	91.4	91.2	90.9
Organic matter, % DM	95.3	95.6	95.1	95.5
Crude protein (CP), % DM	9.6	10.3	9.7	9.8
Starch, % DM	33.9	35.2	35.6	35.7
Crude fat, % DM	2.7	2.5	2.9	2.5
Acid detergent fiber, % DM	21.5	20.3	19.4	20.5
Neutral detergent fiber, % DM	44.0	41.3	40.8	41.1
Neutral detergent insoluble nitrogen, % of total N	3.10	1.56	3.71	2.06
Acid detergent insoluble nitrogen, % of total N	0.60	0.70	0.70	0.80
Rumen degradation (% of total)[u]				
Crude Protein	45.9	57.3	50.1	52.8
Starch	29.0	28.2	69.6	68.8
Energy content[t], DM basis				
Metabolizable energy, Mcal kg^{-1} DM	2.33	2.48	2.33	2.49
Net $energy_m$, Mcal kg^{-1} DM	1.45	1.59	1.46	1.60
Net $energy_g$, Mcal kg^{-1} DM	0.87	0.99	0.87	0.99

[z]RDS = ruminally degradable starch of 28.6 and 69.2% for the low and high RDS treatments, respectively as determined by in situ incubations.

[y]RDP = ruminally degradable protein of 48 and 55% for the low and high RDP treatments, respectively as determined by in situ incubations.

[x]Rumensin premix: 3% monensin sodium or 30,000 mg kg^{-1} monensin sodium

[w]TM Salt: 95% salt,12,000 ppm zinc, 10,000 ppm manganese, 4,000 ppm copper, 400 ppm iodine, 60 ppm cobalt, 30 ppm added selenium.

[v]LS 106: 440,500 IU vitamin A, and 88,000 IU vitamin D_3 kg^{-1}.

[u]Ruminal degradation of CP and starch were estimated by in situ incubation of total mixed rations.

[t]Calculated based on NRC (1996) equations.

Table 4.2 Chemical composition of dietary ingredients

%, DM basis	Whole-shelled corn	Steam-rolled corn	Oat hulls	Fishmeal	Canola meal	Barley
Dry Matter (DM)	85.5	84.2	89.2	96.4	87.5	87.6
Organic Matter	98.5	98.4	95.3	79.8	92.7	96.5
Crude Protein	9.2	8.8	3.3	68.6	41.2	13.1
Ether Extract	4.4	4.3	1.5	11.6	4.4	2.3
Starch	70.0	72.9	5.1	0.2	1.1	53.6

Table 4.3 In situ ruminal degradation kinetics of crude protein (CP) and starch in total mixed rations

	Low RDS[z]		High RDS[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	RDS	RDP	RDS × RDP
CP								
S[w] (%)	34.5	36.9	37.1	34.9	1.20	0.83	0.95	0.12
D[v] (%)	26.3	29.3	45.5	45.8	2.79	<0.01	0.59	0.64
U[u] (%)	39.2	36.7	17.3	19.3	3.42	<0.01	0.64	0.34
Kd[t] (% h^{-1})	5.0	22.1	2.4	3.8	8.51	0.29	0.34	0.41
%BCP[s] (%RUP)	54.1	42.7	49.9	47.3	5.12	0.98	0.24	0.44
%EDCP[r] (%RDP)	45.9	57.3	50.1	52.8	5.12	0.98	0.24	0.44
Starch								
S[w] (%)	14.8	12.2	37.5	40.7	1.44	<0.01	0.83	0.11
D[v] (%)	46.7	50.7	61.3	57.2	9.34	0.32	0.99	0.69
U[u] (%)	38.5	37.2	1.3	2.1	8.33	0.01	0.97	0.90
Kd[t] (% h^{-1})	4.2	4.3	6.7	5.8	2.16	0.41	0.86	0.82
%BS[q] (%RUS)	71.0	71.8	30.4	31.2	4.30	<0.01	0.86	0.99
%EDS[p] (%RDS)	29.0	28.2	69.6	68.8	4.30	<0.01	0.86	0.99

[z]RDS = ruminally degradable starch of 28.6 and 69.2% for the low and high RDS treatments, respectively as determined by in situ incubations.

[y]RDP = ruminally degradable protein of 48 and 55% for the low and high RDP treatments, respectively as determined by in situ incubations.

[x]SEM = pooled standard error of the mean

[w]S = rapidly-degradable fraction (soluble; %)

[v]D = slowly-degradable fraction (potential; %)

[u]U = undegradable fraction (%)

[t]Kd = degradation rate of the D fraction (%h^{-1})

[s]%BCP = percent ruminally-undegradable crude protein

[r]%EDCP = percent effective (ruminally) degradable crude protein (calculated assuming a rumen outflow rate of 6% h^{-1})

[q]%BS = percent ruminally-undegradable starch

[p]%EDS = percent effective (ruminally) degradable starch (calculated assuming a rumen outflow rate of 6% h^{-1})

RDS diet, but no difference was observed on the low RDS diet. The percentage of ADF intake digested in the rumen decreased as RDP level increased on the high RDS diet, but no difference was observed on the low RDS diet (interaction, $P = 0.01$). Total tract digestibility of ADF was greater ($P = 0.03$) on the low RDP diet as compared to the high RDP diet (48.9 vs. 40.9%). Total tract digestibility of starch was greater ($P = 0.02$) on the high RDS diet as compared to the low RDS diet (99.4 vs. 98.2%).

4.4.4 Nitrogen Balance

Nitrogen intake, urinary N output, retention, and N digestibility, were unaffected by dietary treatment ($P > 0.05$; Table 4.6); however, faecal N output tended to increase as dietary RDP level increased on the high RDS diet, but no difference was observed on the low RDS diet (tendency for an interaction, $P = 0.08$). Plasma urea-N concentration tended to increase as dietary RDP level increased on the high RDS diet, but no differences due to RDP level were observed at the low RDS (tendency for an interaction, $P = 0.08$).

Table 4.4 The effects of dietary ruminally-degradable starch (RDS) and ruminally-degradable protein (RDP) levels on ruminal fermentation characteristics in beef heifers

	Low RDS[z]		High RDS[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	RDS	RDP	RDS × RDP
Rumen pH	5.99	5.86	5.81	5.71	0.040	<0.001	0.01	0.59
Ammonia-N, mg dL^{-1}	7.50	9.47	4.97	7.55	1.316	0.01	0.01	0.71
Osmolality, mOsm L^{-1}	258.5	268.8	258.7	279.0	10.06	0.42	0.02	0.44
Volatile fatty acids, m*M*								
Acetate (A)	61.2	57.6	62.5	57.2	3.77	0.91	0.27	0.83
Propionate (P)	16.6	19.0	20.0	21.6	2.62	0.21	0.38	0.84
Butyrate	6.83	6.99	7.29	7.63	0.916	0.56	0.79	0.93
Isobutyrate	0.48	0.50	0.46	0.46	0.030	0.32	0.81	0.67
Valerate	0.55	0.58	0.46	0.46	0.048	0.03	0.68	0.79
Isovalerate	1.17	1.49	1.05	1.08	0.185	0.18	0.36	0.45
Total VFA	86.8	86.2	91.8	88.4	5.93	0.55	0.74	0.82
A:P ratio	3.88	3.19	3.21	2.84	0.375	0.16	0.15	0.65

[z]RDS = 28.6 and 69.2% for the low and high RDS treatments, respectively, as determined by in situ incubations.
[y]RDP = 48 and 55% for the low and high RDP treatments, respectively, as determined by in situ incubations.
[x]SEM = pooled standard error of the mean.

Table 4.5 The effects of dietary ruminally-degradable starch (RDS) and ruminally-degradable protein (RDP) levels on intake, flow to the duodenum, ruminal digestibility, and total tract nutrient digestibility in beef heifers

	Low RDS[z]		High RDS[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	RDS	RDP	RDS × RDP
Dry Matter (DM)								
Intake, kg d^{-1}	10.31	10.49	10.03	10.12	0.759	0.55	0.81	0.93
Flow at the duodenum, kg d^{-1}	5.16	5.18	4.81	5.69	0.563	0.83	0.24	0.26
Digested in the rumen								
kg d^{-1}	4.81	5.37	5.35	4.39	0.377	0.56	0.61	0.08
% of DM intake	49.2	51.2	53.4	43.5	2.02	0.46	0.14	0.05
Total tract digestibility, %	67.5	67.6	70.6	66.3	1.47	0.57	0.22	0.21
Organic Matter (OM)								
Intake, kg d^{-1}	9.82	10.03	9.53	9.66	0.724	0.52	0.75	0.93
Flow at the duodenum, kg/d	4.44	4.48	4.19	4.97	0.434	0.71	0.23	0.28
App. digested in the rumen								
kg d^{-1}	5.05	5.61	5.41	4.67	0.409	0.46	0.82	0.12
% of OM intake	54.7	54.7	56.8	48.4	1.44	0.30	0.07	0.10
Total tract digestibility, %	69.1	69.0	72.1	67.9	1.32	0.51	0.17	0.19
Nitrogen (N)								
Intake, g d^{-1}	156.7	170.3	151.7	157.0	11.76	0.34	0.33	0.66
Flow at the duodenum, g/d	136.8	118.9	125.6	148.5	24.11	0.40	0.81	0.07
App. digested in the rumen								
g d^{-1}	23.0	46.3	29.7	6.2	17.79	0.16	0.99	0.07
% of N intake	16.9	28.4	19.7	5.6	11.52	0.21	0.86	0.13
Total tract digestibility, %	76.7	78.2	78.0	75.1	1.69	0.50	0.58	0.13
Neutral detergent fiber (NDF)								
Intake, kg d^{-1}	4.56	4.42	4.34	4.35	0.395	0.54	0.89	0.56
Flow at the duodenum, kg/d	2.79	2.68	2.45	3.01	0.244	0.98	0.39	0.21
Digested in the rumen								
kg d^{-1}	1.75[a]	1.75[a]	1.86[a]	1.35[b]	0.266	0.14	0.03	0.03
% of NDF intake	37.9[ab]	39.8[a]	42.4[a]	30.3[b]	3.49	0.29	0.06	0.02
Total tract digestibility, %	47.1	41.3	50.0	41.5	2.50	0.54	0.03	0.62

Acid detergent fiber (ADF)								
Intake, kg d^{-1}	2.24	2.19	2.09	2.18	0.194	0.54	0.89	0.53
Flow at the duodenum, kg/d	1.38	1.30	1.20	1.45	0.095	0.91	0.43	0.15
Digested in the rumen								
kg d^{-1}	0.85	0.90	0.89	0.73	0.125	0.29	0.33	0.08
% of ADF intake	36.5^{ab}	41.0^{a}	42.3^{a}	32.9^{b}	3.36	0.55	0.22	0.01
Total tract digestibility, %	47.8	40.4	49.9	41.3	2.76	0.62	0.03	0.85
Starch								
Intake, kg d^{-1}	3.33	3.57	3.51	3.35	0.304	0.94	0.85	0.33
Flow at the duodenum, kg d^{-1}	0.13	0.13	0.11	0.16	0.027	0.60	0.21	0.17
Digested in the rumen								
kg d^{-1}	3.07	3.44	3.39	3.20	0.287	0.85	0.69	0.25
% of Starch intake	96.1	96.3	96.5	95.6	0.71	0.75	0.46	0.28
Total tract digestibility, %	97.8	98.5	99.4	99.4	0.47	0.02	0.38	0.43

[z]RDS = 28.6 and 69.2% for the low and high RDS treatments, respectively, as determined by in situ incubations.
[y]RDP = 48 and 55% for the low and high RDP treatments, respectively, as determined by in situ incubations.
[x]SEM = Pooled standard error of the mean.
[a,b] = Means within a row with different superscripts differ ($P < 0.05$).

Table 4.6 The effects of dietary ruminally-degradable starch (RDS) and ruminally-degradable protein (RDP) levels on nitrogen (N) intake, retention, digestibility, and plasma urea-N concentrations in beef heifers

	Low RDS[z]		High RDS[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	RDS	RDP	RDS × RDP
N intake, g d^{-1}	156.7	170.3	151.7	157.0	11.76	0.34	0.33	0.66
Faecal N, g d^{-1}	37.6	35.3	32.7	39.5	3.56	0.87	0.32	0.08
Urinary N, g d^{-1}	85.1	90.5	81.3	89.8	6.88	0.57	0.11	0.69
UUN[w]:urine-N ratio	0.69	0.78	0.67	0.70	0.050	0.33	0.28	0.56
N retention, g d^{-1}	32.2	45.1	38.0	28.8	6.64	0.50	0.81	0.20
N retained, % of N intake	20.8	24.8	24.5	17.9	3.71	0.68	0.75	0.24
N in faeces, % of N intake	23.3	21.8	22.0	24.9	1.69	0.50	0.58	0.13
N in urine, % of N intake	56.0	53.2	53.6	57.1	3.00	0.81	0.91	0.37
N digestibility, %	76.7	78.2	78.0	75.1	1.69	0.50	0.58	0.13
Plasma Urea-N, mg dL^{-1}	10.6	10.2	8.6	11.4	1.03	0.60	0.19	0.08

[z]RDS = 28.6 and 69.2% for the low and high RDS treatments, respectively, as determined by in situ incubations.
[y]RDP = 48 and 55% for the low and high RDP treatments, respectively, as determined by in situ incubations.
[x]SEM = Pooled standard error of the mean.
[w]UUN = urinary urea-nitrogen

4.4.5 Microbial Protein Production

Total N flow at the duodenum was unaffected by dietary treatment (Table 4.7). Microbial N flow to the duodenum (interaction, $P = 0.04$) and microbial efficiency, expressed as g N kg^{-1} OMTDR (interaction, $P = 0.01$) or g N kg^{-1} TDOMI (interaction, $P = 0.02$), increased as RDP level increased on the high RDS diet but was not affected by RDP level on the low RDS diet. The incorporation of recycled urea-N in microbial N increased as RDP level increased on the high RDS diet, but the opposite was observed on the low RDS diet (interaction, $P = 0.01$). The amount of recycled urea-N incorporated into microbial N, when expressed as a percentage of total microbial N, was greater ($P = 0.04$) on the low RDP diet as compared to the high RDP diet. The amount of recycled urea-N in microbial N (as a percentage of urea-N production) tended to be greater as RDP level decreased on the low RDS diet, but was not different at the high RDS level (tendency for an interaction, $P = 0.06$).

4.4.6 Urea-N Kinetics

Endogenous production of urea-N (UER), urea-N transfer to the GIT (GER), urea-N utilized for anabolism (UUA), urinary urea-N excretion (UUE), and urea-N loss to faeces (UFE) were similar across diets ($P > 0.05$; Table 4.8). The amount of GER that was returned to the ornithine cycle (i.e., ROC) was 23.7% greater in heifers fed the high RDP when compared to those fed the low RDP at the high RDS diet, but ROC was unaffected by RDP level on the low RDS diet (interaction, $P = 0.04$). Fractional transfers of urea-N were unaffected ($P > 0.05$) by dietary treatment.

Table 4.7 The effects of dietary ruminally-degradable starch (RDS) and ruminally-degradable protein (RDP) levels on microbial nitrogen (N) flows at the duodenum measured by ^{15}N as a microbial marker in beef heifers

	Low RDS[z]		High RDS[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	RDS	RDP	RDS × RDP
Duodenal N flow (g N d^{-1})								
Total	106.4	125.6	126.0	149.3	24.11	0.40	0.81	0.07
Microbial	77.9^{ab}	98.7^{a}	76.7^{b}	110.6^{a}	22.48	0.41	0.06	0.04
Microbial efficiency								
g N kg^{-1} OMTDR[w]	15.7^{ab}	14.8^{bc}	11.9^{c}	18.4^{a}	2.25	0.91	0.03	0.01
g N kg^{-1} TDOMI[v]	14.2^{a}	14.0^{a}	10.8^{b}	16.2^{a}	2.24	0.48	0.02	0.02
Recycled urea-N in microbial N								
N, g d^{-1}	10.74^{ab}	7.85^{c}	8.46^{bc}	11.30^{a}	2.643	0.42	0.97	<0.01
% of total microbial N	11.15	7.64	11.01	10.17	1.020	0.18	0.04	0.12
% of urea-N production	7.16^{d}	4.96^{e}	6.44^{de}	6.84^{de}	1.275	0.35	0.18	0.05
% of gut entry of urea-N	12.35	9.17	12.08	11.00	2.272	0.61	0.20	0.45

[z]RDS = 28.6 and 69.2% for the low and high RDS treatments, respectively, as determined by in situ incubations.
[y]RDP = 48 and 55% for the low and high RDP treatments, respectively, as determined by in situ incubations.
[x]SEM = Pooled standard error of the mean.
[w]Microbial N flowing to the duodenum per kg of organic matter (OM) truly digested in the rumen (OMTDR).
[v]Microbial N flowing to the duodenum per kg of total digestible OM intake (TDOMI).
[a-e] = Means within a row with different superscripts differ ($P < 0.05$).

Table 4.8 The effects of dietary ruminally-degradable starch (RDS) and ruminally-degradable protein (RDP) levels on urea-nitrogen (N) recycling kinetics, as measured by continuous jugular infusion of [$^{15}N^{15}N$]-urea in beef heifers

	Low RDS[z]		High RDS[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	RDS	RDP	RDS × RDP
Urea-N fluxes, g d^{-1}								
UER[w]	149.8	149.3	129.6	152.8	6.22	0.23	0.12	0.11
GER[v]	90.7	81.6	72.4	93.5	7.65	0.69	0.47	0.10
UUA[u]	19.9	12.7	14.6	18.0	4.83	0.99	0.71	0.31
ROC[t]	68.3[a]	66.5[ab]	55.5[b]	72.7[a]	3.67	0.40	0.08	0.04
UUE[s]	59.1	67.7	57.2	59.3	2.98	0.13	0.12	0.32
UFE[r]	2.48	2.33	2.30	2.71	0.187	0.61	0.51	0.19
Fractional transfers								
UER to urine	0.39	0.46	0.45	0.39	0.035	0.91	0.90	0.10
UER to GIT[q]	0.61	0.54	0.55	0.61	0.035	0.92	0.90	0.10
GER to ROC	0.76	0.86	0.81	0.79	0.043	0.88	0.36	0.21
GER to faeces	0.03	0.03	0.03	0.03	0.004	0.60	0.98	0.37
GER to UUA	0.22	0.11	0.16	0.18	0.045	0.92	0.39	0.21

[z]RDS = 28.6 and 69.2% for the low and high RDS treatments, respectively, as determined by in situ incubations
[y]RDP = 48 and 55% for the low and high RDP treatments, respectively, as determined by in situ incubations
[x]SEM = Pooled standard error of the mean
[w]UER = Urea-N entry rate
[v]GER = Gastro-intestinal entry rate
[u]UUA = Urea-N utilized for anabolism
[t]ROC = Return to ornithine cycle
[s]UUE = Urinary urea-N excretion
[r]UFE = Urea-N loss to faeces
[q]GIT = Gastro-intestinal tract
[a-b] = Means within a row with different superscripts differ ($P < 0.05$).

4.5 Discussion

In intensive ruminant production systems, N excretion as urea into the environment is a source of pollution and so there is increasing public pressure to improve the efficiency of N utilization in ruminants. Because dietary N intake is a major factor that determines total N intake (Castillo et al. 2000; Yan et al. 2000), lowering dietary N level has been shown to decrease N excretion into the environment in both cattle (Archibeque et al. 2001) and sheep (Kiran and Mutsvangwa 2010). Lowering dietary N levels consistently decreases ruminal NH_3-N concentrations. Because ruminal NH_3-N concentration is negatively correlated with urea-N transfer into the rumen (Kennedy and Milligan 1980), the improved efficiency of N utilization with a lower dietary N level is partly mediated by increased urea-N recycling to the GIT (Lapierre and Lobley 2001). The microbial sequestration of recycled urea-N for microbial protein synthesis depends on the availability of ruminally-fermentable carbohydrate (RFC), and large amounts of recycled urea-N can be reabsorbed into the bloodstream if RFC is limiting. Therefore, it is important to gain an insight into how concomitant manipulation of dietary levels of RDP and RDS in low CP diets would alter urea-N recycling to the GIT and the incorporation of urea-N into microbial protein, which was a major objective of the present study.

Our results show that manipulation of dietary levels of RDP and RDS did not alter UER or GER. A meta-analysis of experimental data on trans-hepatic urea-N fluxes in ruminants at variable N intakes and productive states showed a strong, positive correlation ($r^2 = 0.96$) between N intake and UER (Huntington and Archibeque 1999). Because N intake was not affected by diet in the present study, it is not surprising that UER was similar across diets. When UER:digestible N intake ratios were calculated, they ranged from 1.10 to 1.30, which is within the range of 0.43 to 1.23 reported by Lapierre and Lobley (2001). When compared to previous studies with cattle

fed low CP diets (Marini and Van Amburgh 2003; Wickersham et al. 2008b; Wickersham et al. 2009), our UER estimates are greater and this may be a reflection of the greater N intakes and larger body weights of the heifers used in the present study. The quantity of hepatic urea-N output that was transferred to the GIT (GER), when expressed as a proportion of UER (i.e., GER:UER), ranged from 0.54 to 0.61 and this falls within the range of 0.10 to 0.95 reported by others (Harmeyer and Martens 1980). There is evidence in the literature that providing more RFC by feeding more starch (Kennedy and Milligan 1980; Huntington 1989; Rémond et al. 1996) or by more extensive processing of cereal grains to increase ruminal starch fermentation (Alio et al. 2000; Theurer et al. 2002) increases urea-N transfer to the rumen and microbial protein production. Based on these previous studies, we had anticipated that feeding steam-rolled corn would increase both urea-N transfer to the rumen and UUA (which is assumed to be primarily microbial utilization; Lobley et al. 2000) when compared to feeding whole-shelled corn. In contrast to previous studies, we did not observe any differences in GER or UUA. Even though in situ RDS measurements indicated large differences in RDS between steam-rolled and whole-shelled corn, in vivo measurements of ruminal starch digestion (expressed as absolute amounts or as a proportion of starch intake) were similar. This would indicate that ruminal energy supply was not quantitatively different between heifers fed steam-rolled or whole-shelled corn, which could be a possible explanation for the discrepant results. Other studies (Kiran and Mutsvangwa 2007; Gozho et al. 2008) have also failed to detect differences in GER or UUA when ruminal energy supply was altered via grain processing. Besides ruminal energy supply, ruminal NH_3-N concentration also affects GER (Kennedy and Milligan 1980), possibly because a high ruminal NH_3-N concentration decreases the ruminal epithelium's permeability to urea-N (Egan et al. 1986). Also, high ruminal NH_3-N concentrations decrease bacterial urease activity

(Cheng and Wallace 1979) and, although bacterial urease activity in the rumen was not measured in the present study, Muscher et al. (2010) reported a negative correlation between ruminal urease activity and in vitro serosal-to-mucosal urea flux across ruminal epithelia. Ruminal NH_3-N concentration was lower in heifers fed the high RDS diet when compared with those fed the low RDS diet. Based on the documented effects of ruminal NH_3-N concentration on urea-N transfer, the lower ruminal NH_3-N concentration in heifers fed the high RDS would be expected to have stimulated urea-N transfer to the GIT; however, that was not the case and the reasons for these discrepant results are unclear. It is plausible that the lower ruminal pH that was observed in heifers fed the high RDS when compared with those fed the low RDS diet might have negated any potential stimulatory effects of low ruminal NH_3-N concentration on urea-N transfer. Under the acidotic ruminal conditions that were imposed in heifers fed the high RDS diet, depressed bacterial urease activity might have impaired urea-N transfer to the rumen (Gozho et al. 2008). Using the Ussing chamber technique, Abdoun et al. (2010) varied mucosal pH between 7.4 and 5.4 and observed that serosal-to-mucosal urea flux across ruminal epithelia was maximal at a pH of 6.2 before it declined sharply as mucosal pH was reduced to 5.4. In the present study, ruminal pH in heifers fed the high RDS diet was 5.76 as compared with 5.93 in those fed the low RDS diet. Although this difference was small, we can surmise that the more acidic ruminal conditions with the high RDS diet could have inhibited urea-N transfer to the GIT.

A major goal when feeding ruminants is to optimize ruminal microbial protein supply as it can contribute as much as 60% of the metabolizable protein that is digested in the small intestine (National Research Council 2001). Also, microbial protein synthesis in the rumen offers the only opportunity for anabolic utilization of recycled urea-N that would then benefit the host animal by providing amino acids at the small intestine. Although total N flow at the duodenum

was unaffected by diet, microbial N flow to the duodenum and microbial efficiency both increased as RDP level increased on the high RDS diet, but were not affected by RDP level on the low RDS diet. Data from several studies summarized by Cruz Soto et al. (1994) showed that ruminal fermentation responded to RDP supplementation when dietary RFC was increased, a response that can be attributed to a coupling of energy production with NH_3-N release that, consequently, increases the capture of NH_3-N for microbial protein synthesis. The available evidence indicates that both duodenal flow of microbial N and microbial efficiency are greatest when diets are synchronized for rapid rates of energy and protein degradation (Aldrich et al. 1993; Herrera-Saldana et al. 1990). In diets containing low RDS, supplying additional RDP had minimal positive effects on microbial growth likely because the rates of energy and protein degradation were unsynchronized. Although urea-N transfer can occur across the entire GIT (Lapierre and Lobley 2001), only urea-N that is recycled into the rumen can potentially contribute amino acids to the host animal. In the present study, the amount of recycled urea-N that was recovered as microbial N was low, ranging from 7.85 to 11.30 g d^{-1}. These values are lower when compared to those reported by Wickersham et al. (2008a) (12.3 to 28.9 g d^{-1}) and Wickersham et al. (2008b) (22.9 to 47.4 g d^{-1}). Apart from recycled urea-N, ruminal NH_3-N for microbial use can also be derived from the degradation of dietary RDP. As dietary RDP supply increases, ruminal bacteria become less dependent on recycled urea-N as a source of NH_3-N. In the studies of Wickersham et al. (2008a, 2009b), dietary N intakes (and, therefore, RDP intakes) were much lower than those used in the present study, thus resulting in lower ruminal NH_3-N concentrations and greater rates of urea-N recycling (when expressed as a proportion of UER). Coupled together, lower ruminal NH_3-N concentrations and greater rates of urea-N recycling would, in turn, promote a greater dependence on recycled urea-N as a source of NH_3-N. These

differences could explain the discrepant results between studies in recycled urea-N use for microbial N production.

Most indicators of N utilization (i.e., N intake, retention, and excretion) were not affected by dietary levels of RDS and RDP. Averaged across diets, as a percentage of N intake, 23% of N was excreted in faeces, 55% excreted in urine, and only 22% was retained. Satter et al. (2002) reported that 80 to 90% of N fed to feedlot cattle was excreted with over 50% excreted as urinary N. Over 50% of urinary N was excreted as urea-N which could be readily volatilized to ammonia (Van Horn et al., 1996) and nitrate can leach into the ground (Pakrou and Dillon 1995), thus leading to environmental concerns. On the high RDS diet, increasing dietary RDP level tended to increase faecal N output. This could be due to increased hindgut fermentation leading to a greater recovery of N in microbial protein (Ushida et al. 1991). Microbial protein synthesized in the hindgut cannot be digested or absorbed and is, therefore, excreted in the faeces. Faecal N is considered less of an environmental pollutant than urinary N. Nutritional strategies that partition more excreted N to the faeces than the urine could reduce the negative environmental impact of feedlot production systems.

Only minor dietary effects were observed on ruminal and total tract nutrient digestion. When expressed in absolute amounts or as a percentage of nutrient intake, ruminal digestion of NDF and ADF decreased as RDP level increased on the high RDS diet but no difference was observed on the low RDS diet. The reasons for this interaction are unclear, but could be related to changes in ruminal pH. A ruminal pH below 6.0 suppresses cellulolytic bacterial activity (Ørskov 1986; Owens and Goetsh 1988), thereby decreasing fibre digestion (Hoover 1986; Oliveira et al. 1993). When compared to heifers fed low RDS whose mean ruminal pH was 6.0, heifers fed high RDS had a mean ruminal pH of 5.8, which is below the optimum ruminal pH for

fibre digestion. Although ruminal starch digestion was unaffected, total tract digestibility of starch was greater in heifers fed the high RDS diet as compared with those fed the low RDS diet. In a review of 19 published studies, Theurer et al. (1999) reported a consistent increase in total tract starch digestion when corn grain was processed (i.e., ground, steam-rolled or steam-flaked) when compared with whole-shelled corn. In the present study, however, it should be noted that differences in total tract starch digestion were small and the major site of starch digestion was the rumen as has been reported previously in ruminants fed corn-based diets (Theurer 1986; Theurer et al. 1999). In the present study, we used duodenal sampling and ADIA as an internal marker to estimate duodenal nutrient flows. Other workers (Galloway et al. 1993; Stafford et al. 1996; Heldt et al. 1999; Lehloenya et al. 2008; Wickersham et al. 2008a; Wickersham et al. 2008b) have used ADIA in dairy and beef cattle and it has been established as a reliable method for estimating digesta flow rate (Porter and Sniffen 1986). However, it is recognized that errors associated with sample collection via a T-type duodenal cannula can arise (Harmon and Richards 1997; Titgemeyer 1997), thus leading to erroneous nutrient flows. The reliability of nutrient flow data can be determined by how well it compares with reliable estimates published in the literature and whether it falls within biological limits (Titgemeyer 1997). In the present study, mean OM intake and duodenal OM flow were 9.8 and 4.5 kg d^{-1}, respectively. In comparison, Lehloenya et al. (2008) reported duodenal OM flow of 6.6 kg d^{-1} with an OM intake of 13.7 kg d^{-1} in beef steers weighing an average of 534 kg. Although OM intakes were different between the two studies, OM digested in the rumen (expressed as a percentage of OM intake) was similar (52.1 and 53.7%) and falls within the range of 30 to 60% that has been suggested to be typical for ruminal OM digestion (Titgemeyer 1997). Also, ruminal NDF digestion (mean = 37.6%) represented 84% of total tract NDF digestion. According to Titgemeyer (1997), at least 80% of

total tract NDF digestion should occur in the rumen. Because the proportion of OM and NDF intake that was digested in the rumen in the present study compares favorably with published data (see Titgemeyer 1997), this suggests that ADIA was a suitable marker and that our protocol for duodenal sampling yielded representative digesta samples.

Observed changes in ruminal pH and NH_3-N concentration were typical of those reported when dietary RDS or RDP content is altered. With processed corn, the rapid fermentation of starch in the rumen can lead to increased production of VFA and a reduction in ruminal pH (Owens et al. 1998; Nagaraja and Titgemeyer 2007). However, in the present study, depressed ruminal pH in heifers fed the high RDS diet occurred without any changes in ruminal VFA concentrations. It is possible that the decrease in ruminal pH was due to increased concentrations of other acids such as lactic acid that were not measured. Lactic acid has a lower pKa value than the other VFA and, therefore, has a greater potential of decreasing ruminal pH (Krause and Oetzel 2006; Nagaraja and Titgemeyer 2007). Ruminal pH was also lower in heifers fed high RDP when compared with those fed low RDP, which could be indicative of greater microbial fermentative activity (Cabrita et al. 2006). For all diets, mean ruminal NH_3-N concentration was $\geq$5.0 mg dL^{-1}, which has been suggested to be optimum for maximum microbial protein synthesis (Satter and Slyter 1974). As expected, ruminal NH_3-N concentration was greater in heifers fed high RDP as compared with those fed low RDP, which is in agreement with previous studies in dairy cows (Cunningham et al. 1996; Reynal and Broderick 2005). In the rumen, RDP is broken down through microbial activity to peptides, amino acids and NH_3-N (McAllister et al. 1994). Therefore, a greater concentration of ruminal NH_3-N would be expected when more RDP is fed. Also, ruminal NH_3-N concentration was greater in heifers fed low RDS when compared with those fed high RDS and this could be reflective of reduced microbial proteolytic activity as a

result of the more acidic ruminal environment that was observed in heifers fed high RDS (Van Soest 1994). Microbial sequestration of ruminal NH_3-N during microbial protein synthesis is an energy-dependent process and is most efficient when energy and N availability are coupled (Reynolds and Kristensen 2008), so the lower ruminal NH_3-N concentration in heifers fed high RDS may also be reflective of a more efficient capture of NH_3-N. However, it should be noted that the extent of ruminal starch digestion (and, therefore, ruminal energy supply) and duodenal microbial N supply were unaltered by dietary RDS. Ruminal osmolality was higher for heifers on the high as compared with those on the low RDP diets. Osmolality is a measure of solute concentration in the ruminal fluid (Owens et al. 1998). Higher osmolality values would be expected on high RDP diets with a corresponding higher ruminal NH_3-N concentration. However, normal ruminal osmolality levels are between 240 and 300 mOsm L^{-1} (Owens et al. 1998) and, therefore, all experimental animals were within the normal range.

In summary, when beef heifers were fed a low dietary CP level (10 %), increasing RDP and RDS content increased the amount of urea-N ROC and resulted in greater microbial N supply to the duodenum and improved the efficiency of microbial N production. Incorporation of this feed formulation strategy by producers could be beneficial in terms of reducing overall dietary CP level in beef heifer diets and in reducing the impact of excess N excreted into the environment. Furthermore, judicious combinations of RDP and RDS when feeding low CP diets can potentially enhance the efficiency of microbial N production, which, in turn, can lead to greater animal productivity.

5 EFFECTS OF DIETARY CRUDE PROTEIN AND RUMINALLY-DEGRADABLE PROTEIN LEVELS ON UREA RECYCLING, MICROBIAL PROTEIN PRODUCTION, NITROGEN BALANCE, OMASAL NUTRIENT FLOW, AND MILK PRODUCTION IN LACTATING HOLSTEIN DAIRY COWS

5.1 Abstract

The objective was to determine the effects of dietary crude protein (CP; 14.9 vs. 17.5%) and ruminally-degradable protein (RDP; 63 vs. 69% of CP) content on urea recycling, nitrogen (N) balance, microbial protein production, omasal nutrient flow, and milk production in lactating Holstein cows. Eight multiparous, lactating Holstein cows (4 with ruminal cannulas) were used in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of dietary treatments and 30-d experimental periods. Diet adaptation (d 0-14) was followed by 15 d (d 15-29) of sample and data collection. Jugular infusions of [$^{15}N^{15}N$]-urea (220 mg d^{-1}; 98+ atom percent) were conducted for 4 d (d 26-30) to estimate urea kinetics, with total collection of faeces and urine. Proportions of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea in urinary urea, and ^{15}N enrichment in faeces were used to calculate urea kinetics. Nitrogen intake ($P < 0.01$), and both urinary N ($P < 0.01$) and urea-N ($P < 0.01$) output were greater for cows fed the high compared with those fed the low CP diet. Ruminal ammonia-N concentration tended to be greater in cows fed the high than the low CP diet (20.3 vs. 17.4 mg dL^{-1}; $P = 0.06$), and was greater in cows fed the high RDP as compared with those fed the low RDP diet (21.5 vs. 16.2 mg dL^{-1}; $P < 0.01$). However, N balance, milk yield, and microbial N supply were unaffected ($P > 0.05$) by dietary treatment. Urea-N entry rate (UER) increased ($P < 0.01$) with dietary CP level, but was unaffected ($P > 0.05$) by dietary RDP level. The proportion of urea-N recycled to the GIT (as a percent of UER)

was greater (P = 0.02) in cows fed the low CP compared with those fed the high CP diet. In the short-term, lowering dietary CP level reduced urinary N excretion and had no significant effect on either microbial N supply or milk yield. This resulted in an overall improvement in milk N efficiency.

5.2 Introduction

Current feeding practices often result in lactating dairy cows being fed relatively high crude protein (CP) diets (17.5 to 19.0% CP) in order to support high levels of milk production (NRC 2001). However, overfeeding protein to lactating dairy cows can cause both environmental (Cowling and Galloway 2002; Hristov et al. 2011) and production management (Chandler 1996; Butler 1998; Guo et al. 2004) issues for producers and society. Excess dietary CP is hydrolyzed to ammonia (NH_3) in the rumen, which ultimately is converted to urea in the liver (Lobley et al. 1995) and is subsequently excreted into the environment via the urine (Lapierre and Lobley 2001). Urea is then rapidly hydrolyzed to NH_3 (Van Horn et al. 1996) leading to concerns associated with leaching of nitrates into ground water (Pakrou and Dillon 1995) as well as contributing to air pollution (Hristov et al. 2011). Per unit of feed, protein is a relatively costly nutrient (Chase, et al. 2009). Therefore, the overfeeding of protein and subsequent excretion of excess N represents an inefficient utilization of dietary N (Lobley 1993; Tamminga 1996; Dewhurst et al. 2000) and can increase feed costs (Chandler 1996; Godden et al. 2001; Broderick 2003). Milk urea nitrogen (MUN) is an indicator of the animal's protein status and metabolism (Roseler et al. 1993), and as dietary CP intake increases so does MUN concentration (Canfield et al. 1990). There is evidence that MUN concentrations above 19 mg dL^{-1} lead to a decrease in reproductive function (Butler et al. 1996; Larson et al. 1997) in lactating dairy cows. It is

therefore important to reduce dietary CP levels whilst still maintaining an optimal level of animal production.

Ruminal microorganisms require a balance of fermentable carbohydrate and protein for efficient growth (Nocek and Russell 1988; Hoover and Stokes 1991). Dietary protein is comprised of both ruminally-degradable protein (RDP) and ruminally-undegradable protein (RUP). The RDP portion is broken down into peptides, amino acids and NH_3 which, if sufficient energy is available, are utilized as precursors for microbial protein synthesis (Leng and Nolan 1984; Oldham 1984). Therefore, it is necessary to feed sufficient amounts of RDP to support microbial growth. However, on low CP diets, where RDP supply is insufficient there is opportunity to promote the recycling of urea-N to the gastro-intestinal tract (GIT; Stewart and Smith 2005) and allow incorporation of the N into microbial protein (Lapierre and Lobley 2001). Reducing dietary RDP level has been shown to increase urea recycling to the GIT (Rémond et al. 2009) due to a decrease in ruminal NH_3-N concentration (Kennedy and Milligan 1980). Several studies have investigated the effect of dietary RDP level on urea recycling (Ferrell et al. 2001; Archibeque et al. 2002; Wickersham et al. 2008a, 2009). Increasing RDP intake in steers fed a low quality forage diet lead to an increase in both total endogenous urea-N production and the transfer of urea-N to the rumen (Wickersham et al. 2008a). However, it is unclear how concomitant changes in dietary CP and RDP levels affect urea recycling and microbial protein production in dairy cows fed at higher levels of N intake and with a greater requirement for metabolizable protein.

Optimizing both the level and degradability of dietary CP in high producing dairy cow diets may help lower the excretion of N into the environment, in turn reducing environmental pollution. Through careful manipulation of dietary RDP level on low CP diets, it may be possible

to increase urea-N recycling to the rumen and maintain both microbial protein and milk production of lactating dairy cows typically seen with higher CP diets. The objective of this study was to determine the effects of reduced dietary CP and RDP levels on urea-N recycling, microbial protein production, N balance, omasal nutrient flow, and milk production in lactating dairy cows.

5.3 Materials and Methods

Cows used in this study were cared for in accordance with the guidelines of the Canadian Council of Animal Care (1993) and their use was approved by the University of Saskatchewan Animal Care Committee.

5.3.1 Animals and Experimental Design

Eight multiparous, lactating Holstein cows (711 ± 21 kg of BW; 91 ± 17 days in milk at the start of the study), were used in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of dietary treatments. All cows were housed in individual tie-stalls at the Greenbrae Dairy Research Facility (University of Saskatchewan, Saskatoon, SK, Canada). Four cows were surgically fitted with permanent ruminal cannulas (Bar Diamond, Parma, ID) and used in a metabolism study to determine dietary effects on urea-N recycling, microbial protein production, ruminal fermentation characteristics, N balance, and omasal nutrient flow. Each period consisted of 14 d of dietary adaptation (d 0-14) and 15 d of sample and data collection (d 15-29).

5.3.2 Experimental Treatments and Feeding Management

Experimental diets were composed of a 50:50 forage to concentrate ratio (on a DM basis) and fed to cows as a total mixed ration (TMR) at *ad libitum* intake twice daily at 0900 and 1700. Diets were formulated to contain two levels of CP (14.9 vs. 17.5%, on DM basis) and two levels of RDP (63 vs. 69% of CP). The forage portion of the diet consisted of barley silage and chopped alfalfa hay. Dietary RDP was manipulated by feeding regular canola meal or heated canola meal. All canola meal was purchased from one source (Cargill Ltd, Clavet, SK, Canada). To manipulate ruminally-degradable protein content, a portion of this canola meal was micronized at 160-165^{o}C and held at this temperature for 20 minutes in an insulated container (InfraReady Products Ltd, Saskatoon, SK, Canada). Chemical composition of dietary ingredients is presented in Table 5.1 and ingredients and chemical composition of diets are presented in Table 5.2.

5.3.3 Sample Collection

During each experimental period, individual feed intake and orts remaining were recorded. Samples of TMR were collected on three consecutive days (d 27-29). On d 25, cannulated cows were fitted with temporary vinyl catheters (0.86 mm i.d. × 1.32 mm o.d.; Scientific Commodities Inc., Lake Havasu City, AZ) in both the right and left jugular veins to enable isotope infusion. Both urea-N transfer to the GIT (d 26-30; Lobley et al. 2000) and total-tract N balance (d 26-30) were determined. Samples of urine and faeces were collected on d 26 (prior to the start of [^{15}N^{15}N]-urea infusions) to measure natural background levels of ^{15}N abundance. Starting at 0800 on d 26 of each experimental period, double-labeled urea ([^{15}N^{15}N]-urea, 98+ atom percent excess; Cambridge Isotope Laboratories, MA, USA) dissolved in 2 L of sterile saline solution was continuously infused into the jugular vein at a rate of 220 mg d^{-1} of

[$^{15}N^{15}N$]-urea using a peristaltic pump (Watson and Marlow, Cornwall, UK. Model: 205U) for 96 h. Total faecal and urine output were collected daily for 4 d (d 27-30 of each experimental period). Total daily faecal output for each cow was mixed thoroughly, quantitatively transferred into a plastic container and weighed. A 5% aliquot was taken daily for each cow, composited within period and stored at -20°C. A daily faecal sub-sample was collected to measure ^{15}N. On d 25, indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) were inserted. Urine was collected into a 20 L polyethylene Carboy containing 200 mL of concentrated HCl (VWR Scientific, Mississauga, ON) to maintain urine at a pH < 3. Total daily urine output for each cow was weighed, mixed thoroughly and a 5% aliquot collected. Samples were composited by heifer in each period and stored at -20^{o}C. An additional 50-mL subsample of urine was collected from the composited daily output daily. A 2-mL subsample of urine was collected daily (d 27-30) diluted with 8 mL of distilled water and stored at -20^{o}C.

Omasal digesta flow was determined using, indigestible NDF (iNDF; Reynal et al. 2005), $YbCl_3$ (Siddons et al. 1985), and Cr-EDTA (Udén et al. 1980) as digesta markers for the large particle (LP), small particle (SP), and fluid (FP) phases, respectively. The $YbCl_3$ marker was prepared by dissolving 4.88 g of $YbCl_3 \cdot 6H_2O$ (2.2 g of Yb; Cambridge Isotope Laboratories, Andover, MA) in 1 L of deionised water daily, for each cow. The Cr-EDTA marker solution was infused into the rumen at a rate of 2.7 g d^{-1} of Cr-EDTA. Markers were continually infused into the rumen via the ruminal cannula for 8 d (d 15-23) using a peristaltic pump (Watson and Marlow, Cornwall, UK. Model: 205U). Prior to the start of infusions (d 15), a priming dose equal to one-half of the daily dose of the markers was administered.

Ruminal and omasal samples were collected every 6 h for 48 h (0900, 1500, and 2100 on d 21, 0300, 1200, 1800, and 2400 on d 22 and 0600 on d 23) to represent a 24 h feeding period. Ruminal contents (1 L; 250 mL from the cranial ventral, caudal ventral, central, and cranial dorsal regions) were collected, mixed thoroughly and strained through 4 layers of cheesecloth. Ruminal fluid pH was immediately determined on the filtrate, using a Model 265A portal pH meter (Orion Research Inc., Beverly, MA). A 5 mL aliquot of ruminal fluid was preserved with 1 mL of meta-phosphoric acid (25% wt vol^{-1}), and a second 5 mL aliquot was preserved with 1 mL of 1% sulfuric acid and stored at -20^{o}C. A third 15 mL aliquot was not acidified and was stored at -20^{o}C. Omasal digesta was collected following the procedure of Huhtanen et al. (1997). A 300 mL sample was collected at each sampling time to yield 2.4 L of omasal digesta that was composited by cow within each period and stored at -20^{o}C.

Cows were milked three times a day (0430, 1230, and 1900) and milk weights recorded daily. Milk samples were collected on 3 consecutive days (d 27-29), composited by cow for each day within each period and stored at 4^{o}C.

5.3.4 Sample Analyses

Chemical analysis of dietary ingredients was performed prior to diet formulation, including DM by oven drying at 135°C for 2 h (AOAC 1990; ID no. 930.15), CP using the macro-Kjeldahl procedure (AOAC 1990; ID no. 984.13), soluble CP (Roe et al. 1990), ADF (AOAC 1990; ID no. 973.18), NDF (Van Soest et al. 1991), and ADIN and NDIN (Licitra et al. 1996).

The composited omasal digesta samples were thawed at room temperature and separated by straining through cheesecloth and centrifugation into three phases i.e., LP, SP and FP (Brito et

al. 2006), then freeze-dried (Virtus ‘72, Gardner, New York) and ground in a Braun Aromatic coffee grinder (KSM 2, 2.5 oz). Concentrations of Cr and Yb were determined on a 1 g sample from each of the three phases. Samples were combusted in a muffle furnace at 550^{o}C for 8 h followed by nitric acid digestion as described by Vicente at al. (2004). Chromium concentration was measured by atomic absorption spectrophotometry (Perkin Elmer 2300, Perkin-Elmer Corp, Norwalk, CT) and Yb concentration was measured by atomic emission spectroscopy (Varian Spectra 220, Varian, Mulgrave, Australia). Indigestible NDF was determined on experimental diets and on composited omasal LP and SP phases by ruminal incubation of samples in 5 × 10 cm nylon mesh bags (6 μm pore size; part no. 03-6/5, Sefar America Inc., Depew, NY) for 288 h followed by analysis for NDF (Huhtanen et al. 1994). The concentrations of Cr, Yb, and iNDF in the LP and SP phases, and Cr and Yb concentrations in the FP phase were used to reconstitute the omsasl true digesta (OTD) according to the triple marker method as described by France and Siddons (1986).

Faecal, TMR and ort samples were dried at 55°C for 96 h (Despatch Oven Co., Model V-31) then ground through a 1 mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Total fecal output samples were composited by cow for each period. Total faecal output, OTD, TMR and ort samples were then analyzed for DM by oven drying at 135°C for 2 h (AOAC 1990; ID no. 930.15), OM by ashing at 600^{o}C for at least 8 h (AOAC 1990; ID no. 942.05), CP using the macro-Kjeldahl procedure (AOAC 1990; ID no. 984.13), ether extract (AOAC 1990; ID no. 920.39), ADF (AOAC 1990; ID no. 973.18), and NDF (Van Soest et al. 1991). Amylase and sodium sulfite were used for NDF determination. Total mixed ration samples were also analyzed for ADIN and NDIN (Licitra et al. 1996).

Daily faecal sub-samples were finely ground using a ball mill and prepared for ^{15}N analysis as described by Brito et al. (2006). Briefly, ruminal bacterial, duodenal, and faecal samples containing approximately 100 µg of N were weighed into 5 × 9 mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK). To volatilize NH_3-N, 50 µL of 72 m*M* K_2CO_3 was then added to each tin capsule followed by incubation in a forced-air oven at 60°C for 24 h. Enrichment of ^{15}N in faecal sub-samples was then measured by combustion to N_2 gas in an elemental analyzer and continuous flow isotope ratio-mass spectrometry (Lobley et al. 2000). Urinary urea-N was determined using the diacetyl monoxime method (Procedure No. 0580, Stanbio Laboratory, Boerne, TX) on background and composited 50 mL subsamples of urine (d 26-30) prior to processing for determination of proportions of [$^{15}N^{15}N$]-urea, [$^{14}N^{15}N$]-urea, and [$^{14}N^{14}N$]-urea. Urinary urea was then isolated by applying urine containing 1.5 mg of urea-N to a prepacked cation exchange resin column (AG-50W- × 8 Resin, 100-200 mesh, H+ form; BioRad Laboratories, Hercules, CA) as described by Archibeque et al. (2001). Samples were then eluted with N-free water, air-dried and transferred to borosilicate glass tubes for freeze-drying and then analyzed for the proportions of [$^{15}N^{15}N$]-urea, [$^{14}N^{15}N$]-urea and [$^{14}N^{14}N$]-urea in urinary urea by isotope ratio-mass spectrometry (IRMS: ^{15}N Analysis Laboratory, University of Illinois at Urbana-Champaign). The results were corrected for [$^{14}N^{15}N$]-urea produced by non-molecular reactions (Lobley at al. 2000), and this correction was typically 4 to 6%. Total N in samples of composited daily urine output was determined using the macro-Kjeldahl procedure (AOAC 1990; ID no. 976.05). Diluted daily urine samples were composited by cow for each period (proportional to daily urine output) and analyzed for urinary urea-N (d 26-30) using the colorimetric diacetyl monoxime method (Procedure No. 0580, Stanbio Laboratory, Boerne, TX), and for uric acid (Fossati et al. 1980) and allantoin (Chen and Gomes 1992) (d 26-30). The

urinary excretion of purine derivatives (i.e., uric acid and allantoin) was then used to estimate microbial nonammonia-N (NAN) production (Chen and Gomes 1992).

Ruminal fluid samples preserved with meta-phosphoric acid were analyzed for volatile fatty acid (VFA) concentration (Erwin et al. 1961) using an Agilent 6890 Series Gas Chromatography system (Wilmington, DE) including an Agilent 683 Series injector (5 μL) fitted with a Zebron ZB-FFAP High Performance GC Capillary Column (30.0 m × 320 μm × 0.25 μm, Phenomenex, Torrance, CA). Ruminal fluid samples preserved with sulfuric acid were analyzed for NH_3-N using a phenol-hypochlorite assay (Broderick and Kang 1980). Osmolality was measured on non-acidified ruminal fluid samples using a Vapro™ Vapor Pressure Osmomemter (Model 5520; Wescor Inc., Logan, Utah).

Milk samples were submitted to the Central Milk Testing Lab (Edmonton, AB, Canada) for analysis of protein, fat, lactose, and milk urea nitrogen (MUN) using an infrared analyzer (Milko Scan™ FT 6000, Foss Electric, Denmark) (AOAC 1996; ID no. 972.16).

5.3.5 Calculations and Statistical Analysis

Nitrogen retention was calculated as intake N – faecal N – urinary N – milk N. Apparent digestion of nutrients in the rumen was calculated as nutrient intake – omasal flow of nutrient (kg d^{-1}). Urea-N kinetics was calculated according to the model of Lobley et al. (2000), using urinary enrichment of [$^{15}N^{15}N$]-urea and [$^{14}N^{15}N$]-urea, and total ^{15}N excretion in faeces.

Ruminal fermentation characteristics (pH, NH_3-N, osmolality and VFA concentration) were analyzed using the Proc Mixed repeated measures procedure of SAS (Version 9.1; SAS Institute, Inc. Cary, N. C., 2004). All other data were analyzed as replicated 4 × 4 Latin square design with a factorial arrangement of dietary treatments using the Proc Mixed procedure of SAS

(Version 9.1; SAS Institute, Inc. Cary, N. C. 2004). The following model was used: $Y_{ijkl} = \mu + C_i + P_j + CP_k + RDP_l + (CP \times RDP)_{kl} + \varepsilon_{ijkl}$, where Y_{ijk} = dependent variable, μ = overall mean, C_i = random effect of cow i, P_j = fixed effect of period j, CP_k = fixed effect of dietary CP k, RDP_l = fixed effect of dietary RDP l, $(CP \times RDP)_{kl}$ = fixed effect of the interaction between CP_k and RDP_l, and ε_{ijkl} = random residual error. When significant treatment effects were found, means were compared using the LSD test. Treatment differences were considered significant when $P \leq 0.05$ and tendencies were discussed when $0.05 < P \leq 0.10$.

5.4 Results

5.4.1 Dietary Characteristics

Chemical composition of dietary ingredients is presented in Table 5.1. Soluble crude protein content was lower for heated canola meal as compared with the regular canola meal (16.3 vs. 29.7% of CP) and heated canola meal had a higher ADIN content than the regular canola meal (1.53 vs. 0.73% of N). Dietary ingredients and chemical composition are presented in Table 5.2. Chemical analysis of TMR indicated that CP levels were 14.9 and 17.5% CP (on DM basis).

5.4.2 Ruminal Fermentation Characteristics

Daily average ruminal pH was unaffected ($P > 0.05$) by dietary treatment (Table 5.3). Ruminal NH_3-N concentration tended ($P = 0.06$) to be greater in cows fed the high compared with the low CP diet (20.3 and 17.4 mg dL^{-1}, respectively), and was greater ($P < 0.01$) in cows fed the high compared with the low RDP diet (21.5 and 16.2 mg dL^{-1}). Ruminal osmolality was unaffected ($P > 0.05$) by dietary treatment. Individual VFA concentrations, total VFA concentration, and acetate:propionate ratio were unaffected ($P > 0.05$) by dietary treatment,

Table 5.1 Chemical composition of dietary ingredients

	DM[z]	CP[y]	SCP[x]	ADF[w]	NDF[v]	ADIN[u]	NDIN[t]
Barley silage	35.9	11.2	69.2	29.2	52.2	0.47	1.07
Alfalfa hay	92.8	14.1	39.5	35.7	51.0	1.17	2.18
Canola meal	88.1	41.0	29.7	21.7	31.6	0.73	1.95
Heated canola meal[s]	97.7	41.0	16.3	24.0	41.0	1.53	5.21
Barley grain	88.8	10.34	28.3	6.4	25.0	0.29	1.76

[z]DM = dry matter, %
[y]CP = crude protein, % DM
[x]SCP = soluble crude protein, % of CP
[w]ADF = acid detergent fiber, % DM
[v]NDF = neutral detergent fiber, % DM
[u]ADIN = acid detergent insoluble nitrogen (N), % N
[t]NDIN = neutral detergent insoluble nitrogen, % N
[s]Heated canola meal = canola meal micronized at 160-165^{o}C and held at this temperature for 20 minutes

Table 5.2 Ingredient and chemical composition of diets fed to lactating Holstein dairy cows

	Low CP[z]		High CP[z]	
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]
Total mixed diet, % DM basis				
Barley Silage	34.6	34.6	34.4	34.4
Alfalfa Hay	14.6	14.6	14.5	14.5
Barley Grain	38.3	38.3	30.8	30.8
Canola meal	0.0	10.2	0.0	18.1
Heated Canola Meal	10.2	0.0	18.1	0.0
U of S Premix[x]	1.6	1.6	1.5	1.5
MagSulf7H_2O[w]	0.1	0.1	0.1	0.1
Limestone[v]	0.4	0.4	0.4	0.4
Sodium Bicarbonate[u]	0.3	0.3	0.3	0.3
Chemical composition of total mixed diet				
Dry matter (DM), %	46.3	46.5	45.4	46.8
Organic matter, % of DM	92.0	92.0	91.2	91.5
Crude protein (CP), % of DM	14.9	14.9	17.5	17.5
RDP, % of CP	65.1	69.8	61.0	68.0
Crude fat, % DM	2.6	2.5	2.8	2.6
Acid detergent fiber, % DM	22.9	21.8	25.3	24.2
Neutral detergent fiber, % DM	36.3	35.6	38.6	37.0
Neutral detergent insoluble nitrogen (N), % of total N	2.3	1.4	2.9	1.4
Acid detergent insoluble nitrogen, % of total N	1.8	1.1	2.6	1.6
NE_L[t], Mcal/kg of DM	1.48	1.45	1.50	1.49

[z]CP = Crude protein at 14.9 and 17.5% for the low and high CP treatment, respectively

[y]RDP = Ruminally degradable protein at 63 and 69% of CP for the low and high RDP treatments, respectively as calculated from CPM-Dairy

[x]U of S Premix: 16.0 %DM Ca, 8.0 %DM P, 4.5 %DM Mg, 1.8 %DM K, 1.0 %DM S, 6.3 %DM Na, 10.0 %DM Cl, 2100.0 mg/kg Fe, 2160.0 mg/kg Zn, 675.0 mg/kg Cu, 1120.0 mg/kg Mn, 17.0 mg/kg Se, 16.0 mg/kg Co, 40.0 mg/kg I, 240.0 KIU/kg Vitamin A, 50.0 KIU/kg Vitamin D and 1500.0 IU/kg Vitamin E

[w]MagSulf7H2O: 9.85 %DM Mg and 13.0 %DM S

[v]Limesonte: 33.0 %DM Ca, 0.02 %DM P, 2.1 %DM Mg, 0.12 %DM K, 0.04 %DM S, 0.06 %DM Na, 0.03 %DM Cl and 3500.0 mg/kg Fe

[u]Sodium Bicarbonate: 27.0 %DM Na

[t]Net energy of lactation, calculated from CPM-Dairy

Table 5.3 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on ruminal fermentation characteristics in lactating Holstein dairy cows

	Low CP[z]		High CP[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	CP	RDP	CP × RDP
Ruminal pH	5.90	5.93	5.91	5.99	0.049	0.44	0.26	0.58
Ammonia-N, mg dL^{-1}	14.5	20.2	17.8	22.8	1.45	0.06	<0.01	0.81
Osmolality, mOsmol L^{-1}	231.9	218.3	226.9	233.0	11.95	0.69	0.76	0.43
Volatile fatty acids, m*M*								
Acetate (A)	48.0	48.2	49.4	48.8	1.54	0.15	0.83	0.58
Propionate (P)	23.4	20.7	21.4	21.7	2.45	0.85	0.63	0.56
Butyrate	9.0	9.9	10.1	9.5	0.95	0.69	0.91	0.44
Valerate	1.5	1.2	1.2	1.4	0.26	0.88	0.81	0.44
Isobutyrate	0.59	0.63	0.59	0.64	0.046	0.78	0.04	0.86
Isovalerate	0.68	0.80	0.73	0.78	0.074	0.67	0.03	0.39
Total VFA	83.1	81.4	83.5	82.8	1.52	0.54	0.43	0.73
A:P ratio	2.25	2.42	2.42	2.47	0.262	0.48	0.47	0.68

[z]CP = 14.9 and 17.5% for the low and high CP treatments, respectively

[y]RDP = 63 and 69% of CP for the low and high RDP treatments, respectively as calculated from CPM-Dairy

[x]SEM = Pooled standard error of the mean

except that isobutyrate and isovalerate concentrations were greater ($P < 0.05$) in cows fed the high as compared with the low RDP diet.

5.4.3 Nutrient Intake, Omasal Nutrient Flow, and Ruminal and Total-Tract Nutrient Digestibility

A CP × RDP interaction was detected for DM and OM apparently digested in the rumen expressed as kg d^{-1} or as a percent of nutrient intake (Table 5.4). On the low CP diet, both DM ($P \leq 0.02$) and OM ($P \leq 0.03$) digested in the rumen were greater on the high as compared with the low RDP diet, but no difference due to dietary RDP was observed on the high CP diet. Total-tract OM digestibility was unaffected ($P > 0.05$) by dietary treatment. Organic matter flow at the omasum was greater ($P < 0.05$) for cows fed the high as compared with the low CP diet but was unaffected ($P > 0.05$) by dietary RDP level. An interaction was detected for NDF ($P = 0.02$) intake. For the low CP diet, NDF intake was greater ($P = 0.02$) on the high as compared with the low RDP diet. The opposite was observed on the high CP diet. A similar trend ($P = 0.07$) was observed for ADF intake. Both NDF and ADF flow at the omasum were greater ($P < 0.01$) on the high as compared with the low CP diet. Both NDF and ADF apparently digested in the rumen (kg d^{-1} or as a percent of nutrient intake), and total-tract digestibility were greater ($P < 0.05$) for the low as compared with the high CP diet.

5.4.4 Nitrogen Balance

Nitrogen intake ($P < 0.01$), and both urinary N ($P < 0.01$) and urea-N ($P < 0.01$) output were lower in cows fed the low CP diets as compared with those fed the high CP diet (Table 5.5). On the high CP diet, faecal N output was greater on the low as compared with the high RDP

Table 5.4 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on intake, flow at the omasum, ruminal digestibility, and total-tract digestibility in lactating Holstein dairy cows

	Low CP[z]		High CP[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	CP	RDP	CP × RDP
Dry matter (DM)								
Intake, kg d^{-1}	25.5[b]	27.0[ab]	27.0[a]	25.2[b]	0.80	0.83	0.77	0.03
Flow at the omasum, kg d^{-1}	15.0	13.8	15.1	15.5	0.91	0.13	0.40	0.16
Digested in the rumen								
kg d^{-1}	10.4[b]	13.0[a]	11.8[ab]	10.0[b]	0.72	0.23	0.53	0.01
% of DM intake	41.1[b]	48.5[a]	44.0[ab]	38.8[b]	2.69	0.14	0.59	0.02
Total-tract digestibility, %	69.5	67.9	68.2	67.7	1.24	0.52	0.40	0.66
Organic matter (OM)								
Intake, kg d^{-1}	23.2	24.7	24.8	23.3	0.67	0.85	0.99	0.05
Flow at the omasum, kg d^{-1}	11.5	10.9	12.0	12.2	0.65	0.03	0.52	0.28
Digested in the rumen								
kg d^{-1}	11.9[b]	13.7[a]	12.7[ab]	11.2[b]	0.59	0.16	0.76	0.02
% of OM intake	50.9[ab]	55.6[a]	51.7[ab]	47.6[b]	2.08	0.05	0.83	0.03
Total-tract digestibility, %	71.6	70.1	70.4	70.1	1.17	0.61	0.45	0.61
Neutral detergent fiber (NDF)								
Intake, kg d^{-1}	9.4[ab]	10.1[a]	9.8[ab]	9.2[b]	0.40	0.34	0.88	0.02
Flow at the omasum, kg d^{-1}	3.3	3.3	4.3	3.9	0.26	<0.01	0.31	0.23
Digested in the rumen								
kg d^{-1}	6.1	6.7	5.6	5.3	0.47	0.03	0.51	0.23
% of NDF intake	64.9	67.4	56.3	58.0	3.01	0.01	0.42	0.88
Total-tract digestibility, %	68.9	68.3	66.1	64.8	1.18	<0.01	0.30	0.74
Acid detergent fiber (ADF)								
Intake, kg d^{-1}	6.1	6.3	6.2	5.9	0.34	0.23	0.63	0.07
Flow at the omasum, kg d^{-1}	1.6	1.7	2.2	2.1	0.13	<0.01	0.87	0.13
Digested in the rumen								
kg d^{-1}	4.5	4.6	4.1	3.9	0.33	0.01	0.83	0.31

% of ADF intake	72.7	72.8	65.3	65.3	2.36	<0.01	0.96	0.97
Total-tract digestibility, %	22.4	18.4	15.1	14.3	3.86	0.02	0.25	0.42

[z]CP = 14.9 and 17.5% for the low and high CP treatments, respectively
[y]RDP = 63 and 69% of CP for the low and high RDP treatments, respectively as calculated from CPM-Dairy
[x]SEM = Pooled standard error of the mean
[a,b] = Means within a row with different superscripts differ ($P < 0.05$)

Table 5.5 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on nitrogen (N) balance, and digestibility in lactating Holstein dairy cows

	Low CP[z]		High CP[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	CP	RDP	CP × RDP
N intake, g d^{-1}	608.4	643.3	752.7	718.7	24.56	<0.01	0.98	0.13
Faecal output, kg DM d^{-1}	7.7[b]	8.6[a]	8.6[a]	8.1[ab]	0.24	0.54	0.38	0.01
Faecal N, g d^{-1}	168.2[b]	189.4[b]	215.5[a]	180.7[b]	11.01	0.03	0.37	<0.01
Urine output, kg d^{-1}	21.3	22.6	23.3	24.6	1.43	0.06	0.19	0.93
Urinary N, g d^{-1}	152.8	169.7	248.5	263.9	16.20	<0.01	0.37	0.97
UUN:urine-N ratio	0.90	0.93	0.78	0.74	0.151	0.39	0.97	0.85
Milk N, g d^{-1}	171.1	173.7	176.9	180.6	7.16	0.24	0.54	0.92
N retention, g d^{-1}	112.0	106.4	126.7	87.1	22.95	0.93	0.38	0.50
Milk N efficiency, % of intake	27.7	27.7	23.6	24.7	1.08	<0.01	0.57	0.53
N digestibility, %	72.3	70.4	71.3	74.4	1.89	0.39	0.74	0.17

[z]CP = 14.9 and 17.5% for the low and high CP treatments, respectively

[y]RDP = 63 and 69% of CP for the low and high RDP treatments, respectively as calculated from CPM-Dairy

[x]SEM = Pooled standard error of the mean

diet, but no difference was observed on the low CP diet (interaction, $P < 0.01$). Milk N output, N retention, and N digestibility were not affected ($P > 0.05$) by dietary treatment. Milk N efficiency was greater ($P < 0.01$) for cows on the low as compared with those on the high CP diet, but was unaffected ($P > 0.05$) by dietary RDP level.

5.4.5 Microbial Protein Production

Microbial N supply averaged 386.5 g microbial N d^{-1} and did not differ ($P > 0.05$) amongst dietary treatments (Table 5.6).

5.4.6 Milk Production and Composition

Milk yield and yields of protein and lactose were unaffected ($P > 0.05$) by dietary treatment (Table 5.7). On the low CP diet, milk fat yield was greater for cows fed the low as compared with those fed the high RDP diet but was unaffected by RDP level on the high CP diet (interaction, $P = 0.05$). Milk urea nitrogen (MUN) was greater ($P < 0.01$) on the high as compared with the low CP diet (17.8 and 12.7 mg dL^{-1}, respectively). On the high CP diet, MUN was greater for cows fed the high as compared with those fed the low RDP diet, but no difference was observed between RDP levels fed the low CP diet (interaction, $P < 0.01$). Milk fat, protein and lactose contents were unaffected ($P > 0.05$) by diet.

Table 5.6 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on microbial nitrogen (N) flow at the omasum measured by urinary excretion of purine derivatives (PD) in lactating Holstein dairy cows

	Low CP[z]		High CP[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	CP	RDP	CP × RDP
Urinary excretion								
Total output, kg d^{-1}	21.3	22.6	23.3	24.6	1.43	0.06	0.19	0.93
Allantoin, mmol d^{-1}	452.4	519.7	490.9	495.5	16.65	0.71	0.10	0.14
Uric acid, mmol d^{-1}	47.7	54.6	58.1	51.7	5.87	0.45	0.95	0.20
Milk PD[w], mmol d^{-1}	32.9	33.6	33.6	33.6	2.70	0.57	0.49	0.52
Total PD, mmol d^{-1}	532.9	607.9	582.6	580.8	19.94	0.63	0.15	0.14
Microbial N[v] $supply^{-1}$								
g microbial N d^{-1}	355.2	409.4	391.3	390.0	14.44	0.63	0.16	0.14
g microbial N kg^{-1} DOMR[u]	36.6	40.3	38.4	42.1	2.35	0.45	0.15	0.98

[z]CP = 14.9 and 17.5% for the low and high CP treatments, respectively

[y]RDP = 63 and 69% of CP for the low and high RDP treatments, respectively as calculated from CPM-Dairy

[x]SEM = Pooled standard error of the mean

[w]Estimated as 1 mmol L^{-1} milk (i.e. 1 × milk yield (L d^{-1})) from Chen and Gomes (1992)

[v]Microbial N supply was calculated according to Chen and Gomes (1992)

[u]Digestible organic matter in the rumen (DOMR) was calculated as 0.65 x digestible OM intake (Chen and Gomes 1992)

Table 5.7 The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on milk production and composition in lactating Holstein dairy cows

	Low CP[z]		High CP[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	CP	RDP	CP × RDP
Milk and component yield								
Milk, kg d^{-1}	38.3	38.5	39.0	39.4	1.22	0.26	0.68	0.89
3.5% FCM[w], kg d^{-1}	39.3	38.4	39.4	39.4	0.71	0.15	0.25	0.18
Fat, kg d^{-1}	1.43[a]	1.30[b]	1.40[a]	1.38[a]	0.037	0.27	0.01	0.05
Protein, kg d^{-1}	1.25	1.30	1.25	1.30	0.046	0.54	0.54	0.54
Lactose, kg d^{-1}	1.73	1.75	1.78	1.80	0.064	0.21	0.51	1.00
MUN[v], mg dL^{-1}	12.3[c]	13.0[c]	16.0[b]	19.5[a]	0.93	<0.01	<0.01	<0.01
Milk components								
Fat, %	3.68	3.53	3.60	3.50	0.158	0.66	0.29	0.82
Protein, %	3.30	3.25	3.28	3.28	0.118	1.00	0.70	0.70
Lactose, %	4.56	4.55	4.50	4.52	0.072	0.20	0.75	0.72

[z]CP = 14.9 and 17.5% for the low and high CP treatments, respectively

[y]RDP = 63 and 69% of CP for the low and high RDP treatments, respectively as calculated from CPM-Dairy

[x]SEM = Pooled standard error of the mean

[w]FCM = Fat corrected milk

[v]MUN = Milk urea nitrogen

[a,b] = Means within a row with different superscripts differ ($P < 0.05$)

5.4.7 Urea-N Kinetics

Urea- N entry rate (UER) increased ($P < 0.01$) with dietary CP level, but was unaffected ($P > 0.05$) by RDP level (Table 5.8). Urea-N utilized for anabolism (UUA) tended ($P = 0.07$) to decrease as dietary CP level increased, but was unaffected by dietary RDP level. Urinary urea-N excretion (UUE) increased ($P < 0.01$) as dietary CP level increased, but was unaffected by dietary RDP level. Gastro-intestinal entry rate (GER) and urea-N loss to faeces (UFE) were unaffected ($P > 0.05$) by dietary treatment. The proportion of urea-N utilized for anabolic purposes (as a percent of urea-N returned to the GIT; GER to UUA) tended ($P = 0.06$) to be greater in cows fed the low when compared with those fed the high CP diet. Consequently, the proportion of urea-N returned to the ornithing cycle (ROC) was lower ($P = 0.05$) in cows fed the low as compared with those fed a high CP level. Fractional transfers of urea-N were unaffected ($P > 0.05$) by dietary RDP level; however, the proportion of UER excreted in the urine increased ($P = 0.02$) as dietary CP level increased. Conversely, the proportion of UER partitioned to the GIT decreased ($P = 0.02$) as dietary CP level increased. The proportion of GER from ROC increased ($P = 0.03$) as dietary CP level increased, but the proportion of GER voided in the faeces was unaffected ($P > 0.05$) by dietary CP level.

Table 8. The effects of dietary crude protein (CP) and ruminally-degradable protein (RDP) levels on urea-nitrogen (N) recycling kinetics, as measured by continuous jugular infusions of [$^{15}N^{15}N$]-urea in lactating Holstein dairy cows

	Low CP[z]		High CP[z]			*P* values		
Item	Low RDP[y]	High RDP[y]	Low RDP[y]	High RDP[y]	SEM[x]	CP	RDP	CP × RDP
Urea-N fluxes, g d^{-1}								
UER[w]	405.1	436.5	502.0	495.1	26.61	<0.01	0.54	0.35
GER[v]	279.0	292.9	309.3	287.2	23.30	0.50	0.82	0.34
UUA[u]	59.0	51.4	46.7	24.0	10.29	0.07	0.14	0.43
ROC[t]	213.3	230.4	251.0	252.1	16.39	0.05	0.47	0.52
UUE[s]	129.0	146.7	193.1	201.6	13.71	<0.01	0.38	0.76
UFE[r]	9.0	12.8	9.2	9.2	1.59	0.26	0.23	0.22
Fractional transfers								
UER to urine	0.31	0.33	0.40	0.41	0.027	0.02	0.45	0.84
UER to GIT[q]	0.69	0.67	0.60	0.56	0.027	0.02	0.45	0.84
GER to ROC	0.76	0.78	0.85	0.88	0.029	0.03	0.47	0.91
GER to faeces	0.03	0.05	0.03	0.03	0.008	0.27	0.16	0.12
GER to UUA	0.21	0.17	0.12	0.09	0.034	0.06	0.36	0.94

[z]CP = 14.9 and 17.5% for the low and high CP treatments, respectively
[y]RDP = 63 and 69% of CP for the low and high RDP treatments, respectively as calculated from CPM-Dairy
[x]SEM = Pooled standard error of the mean
[w]UER = Urea-N entry rate
[v]GER = Gastro-intestinal entry rate
[u]UUA = Urea-N utilized for anabolism
[t]ROC = Return to ornithine cycle
[s]UUE = Urinary urea-N excretion
[r]UFE = Urea-N loss to faeces
[q]GIT = Gastro-intestinal tract

5.5 Discussion

Dairy cows capture only 25 to 35% of their dietary CP intake as milk protein (Chase et al. 2009); therefore, large amounts of dietary N are excreted, potentially leading to environmental pollution (Cowling and Galloway 2002) and a decrease in the efficiency of N utilization. Reducing dietary N intake reduces overall N excretion into the environment (Archibeque et al. 2001) but has also been shown to reduce milk production (Kung and Huber 1983). Lowering dietary RDP level reduces ruminal NH_3 concentration which, in turn, increases urea-N transfer to the rumen (Kennedy and Milligan 1980). The recycling of urea-N to the GIT is an opportunity to improve the efficiency of N utilization and provide a source of N for microbial protein production (Lapierre and Lobley 2001). Therefore, this study employed nutritional strategies to investigate the influence of simultaneous changes in the dietary content and degradability of CP on urea-N recycling, N balance, microbial protein production, and milk production in lactating Holstein dairy cows.

Endogenous urea-N production in the present study averaged 459.7 g d^{-1} which is similar to values (average 388.2 g d^{-1}) reported by Gozho et al. (2008) in dairy cows. Results show that UER increased with increasing N intake which is in agreement with others studies (Archibeque et al. 2001; Marini and Van Amburgh 2003; Marini et al. 2004). Endogenous urea-N production is a consequence of ruminal microbial degradation of dietary protein to NH_3 (Bach et al. 2005) and the subsequent need for excess NH_3 to be detoxified by the liver through conversion to urea (Stewart and Smith 2005). Therefore, higher UER values on higher CP diets is expected. A positive correlation between N intake and UER values has been reported by Huntington and Archibeque (1999). The UER:digestible N intake ratios ranged from 0.92 to 0.96 and are similar to those reported by Lapierre and Lobley (2001). The high ratios observed in the present study

show that large amounts of N passed through the urea-N pool daily, indicating that urea-N recycling is important in maintaining a positive N balance in dairy cows. The transfer of hepatic urea-N output to the GIT (GER) was unaffected by dietary treatment; however, when the GER was expressed as a proportion of UER the value was greater for the 14.9% CP diet as compared with the 17.5% CP diet, and ranged from 0.56 to 0.69 which is within the range of previous reports of 0.10 to 0.95 (Harmeyer and Martens 1980). The transfer of hepatic urea-N output to the GIT can be influenced by several factors, including dietary protein level (Marini et al. 2004; Wickersham et al. 2009), forage to concentrate ratio (Huntington et al. 1996), dry matter intake (Sarraseca et al. 1998), and diet digestibility (Theurer et al. 2002). In particular, it has been shown that an increase in ruminal NH_3-N concentration can lead to a reduction in GER (Kennedy and Milligan 1980). This could be due to the negative influence of high NH_3-N concentration on both the ruminal epithelium's permeability to urea-N (Egan et al. 1986) and ruminal urease activity (Cheng and Wallace 1979). However, more recently, in vitro studies by Abdoun et al. (2006) demonstrated a pH-dependent effect of ammonia on urea flux. The greater proportion of endogenous urea-N transferred to the GIT on the 14.9% CP diet can therefore be explained by lower ruminal NH_3-N concentration as compared with the 17.5% CP diet. Recycled urea-N would therefore provide additional N that could potentially be used for microbial protein production. The amount of GER used for anabolic purposes (UUA) tended to be greater for the 14.9% CP diet as compared with the 17.5% diet. Kiran and Mutsvangwa (2010) also noted an increase in utilization of UUA from GER in lambs fed a 10% as compared with a 15% CP diet (GER to UUA; 0.526 vs. 0.384). Lobley et al. (2000) reported that UUA is utilized for amination and transamination reactions but that the major use of UUA is the sequestration of NH_3-N into microbial protein. The utilization of recycled urea-N for microbial protein production offers an

opportunity to improve the efficiency of N utilization in ruminants. The tendency for an increase in UUA, on the low (14.9%) CP diet, may have aided in the maintenance of microbial protein in the present study.

Microbial protein supply averaged 386.5 g microbial N d^{-1} however microbial N supply values reported in the literature are variable. Colmenero and Broderick (2006) fed diets ranging from 13.5 to 19.4% CP (DM basis) to lactating Holstein cows and reported an average value of 455 g microbial N d^{-1} for microbial production. Cunningham et al. (1996) reported an average value of 223 g microbial N d^{-1} for dairy cows fed dietary CP levels ranging from 16.5 to 18.5%. These data demonstrate that microbial protein production can vary across different dietary treatments and between studies but values from the present study fall within the reported range.

Microbial protein is an important source of amino acids for the ruminant, with an amino acid profile closely matching that of both meat and milk (Ørskov 1992) and can contribute 60 to 80% of the metabolizable protein supply reaching the small intestine (NRC 2001). Dietary protein is degraded in the rumen to peptides, amino acids and NH_3-N which are important precursors for microbial growth (Wallace 1997). It has been found that ruminal NH_3-N concentrations below 5 mg dL^{-1} could limit microbial protein production (Satter and Slyter 1974; Russell and Strobel 1987). Ruminal NH_3-N concentrations tended to decrease as dietary CP level decreased from 17.5% to 14.9% CP (20.3 vs. 17.4 mg dL^{-1}) but were above the critical threshold reported earlier. Other studies in dairy cows have reported a decrease in ruminal NH_3-N concentrations with a reduction in dietary CP level (Christensen et al. 1993; Cunningham et al. 1996; Reynal and Broderick 2005). Reynal and Broderick (2005) observed a reduction in ruminal NH_3-N concentration from 12.0 to 7.2 mg dL^{-1} with an approximate 1% unit decrease in dietary CP level (18.6 to 17.5%, respectively). The tendency for ruminal NH_3-N levels to

decrease on the 14.9% CP diet as compared with the 17.5% CP diet affected urea-N recycling to the GIT. The increase in UER to GIT on the 14.9% CP diet, as compared with the 17.5% CP diet compensated for the decreased dietary CP level. This could explain why microbial protein supply was maintained the same on the 14.9% CP diets as on the 17.5% CP diets. The salvage of N via urea-N recycling (Stewart and Smith 2005) represents an opportunity to improve the efficiency of feed N utilization and can lead to a reduction in the loss of N to the environment.

As expected, cows fed the 14.9% diet consumed less N than the cows fed the 17.5% CP diet. Furthermore, N retention averaged 108.1 g d^{-1} but did not differ across dietary treatments. Nitrogen retention values in the present study are higher than the 28.3 g d^{-1} reported by Castillo et al. (2001). This discrepancy may be accounted for by the lower N intakes reported by Castillo et al. (2001) as compared with our experimental cows (468.8 vs. 680.8 g d^{-1}, respectively). However, a review of N balance data from 35 published studies summarizing 125 different diets by Spanghero and Kowalski (1997) reported on several factors that can lead to either the underestimation or overestimation of N retention values. For example, the incomplete collection or volatile losses of NH_3-N during collection or subsequent drying of faeces can lead to the underestimation of faecal N (Spanghero and Kowalski 1997).

Furthermore, feeding the 14.9% CP diet decreased urinary N excretion by 37% when compared to feeding the 17.5% CP diet. Other studies have reported similar findings (Archibeque et al. 2001; Marini and Van Amburgh 2003; Davies et al. 2013). Excess N is excreted in both the faeces and urine. However, urinary N is composed of mainly urea (Broderick 2003) and in this study between 76.4 and 86.4% of urinary N was excreted as UUN. This represents an irreversible loss of N to the cow. Urinary urea-N is also more damaging to the environment than faecal N, as it can be rapidly volatilized to NH_3 (Van Horn et al. 1996) in the

presence of bacterial urease (McGinn et al 2002). This can lead to the pollution of both ground water and air (Pakrou and Dillon 1995; Hristov et al. 2011). Therefore, a reduction in N intake can be beneficial in terms of environmental stewardship related to intensive farming practices. Feeding the 14.9% CP diet also improved milk N efficiency (MNE) by 13% when compared with feeding the 17.5% CP diet (27.7% vs. 24.2% of intake, respectively). Milk N efficiency is calculated as the quantity of N secreted in milk expressed as a proportion of feed N intake and is a commonly-used as an index for assessing the efficiency of conversion of feed into milk N. Broderick (2003) reported a 30.3% MNE when feeding a 15.1% CP diet and a subsequent decline to 27.0% when dietary CP level was increased to 16.7%. On average, milk yield was 38.8 kg/d and did not differ amongst diets, demonstrating that, at least in the short-term, cows fed 14.9% CP can maintain similar levels of milk yield when compared to cows fed 17.5% CP. The average for the Greenbrae Dairy Research Herd (University of Saskatchewan) is 39.1 kg d^{-1}; therefore, the performance of cows in this study was similar to that of other cows in the herd. In agreement with these findings, Colmenero and Broderick (2006) fed diets containing 13.5 to 19.4% CP and reported average milk yields ranging from 36.3 to 38.8 kg d^{-1} with no difference between dietary treatments. The ability to maintain milk yield in the present study could be a result of the maintenance of microbial protein production in this short-term study. Maintaining milk production levels with a reduction in dietary N input not only improves MNE but also reduces overall feed costs. However, further research is needed to define production throughout a full lactation and document any potential influence a reduction in dietary CP level may have on reproductive efficiency.

Ruminal NH_3-N decreased as dietary RDP level decreased from high to low (16.2 vs. 21.5 mg dL^{-1}, respectively). Dietary RDP level was manipulated by inclusion of canola meal

and/or heated canola meal in the diet. Canola meal is a commonly used feed ingredient in western Canada and is an excellent source of protein in terms of matching the amino acid requirements of milk production (NRC 2001). The ruminal degradability of canola meal can be altered through treatment with heat (McKinnon et al. 1991; McKinnon et al. 1995). As seen in Table 2, both the soluble crude protein and acid detergent insoluble nitrogen levels were altered by heating canola meal and explain the differences in ruminal NH_3-N concentrations for the low and high RDP diets.

In summary, ruminal NH_3-N concentrations were altered by lowering diet CP and RDP levels. However, increases in the fractional transfer of UER to the GIT compensated for reduced ruminal NH_3-N concentrations and allowed for the maintenance of microbial protein supply, in this short-term study. Even though both dietary CP and RDP levels were reduced, this did not result in reduced milk yield. In addition, as dietary CP level was reduced, there was also a decline in N excretion via the urine. Lowering N excretion not only improves N efficiency, but also reduces the negative environmental impact of such intensive farming systems. There are also potential economic benefits to lowering the level of CP in the diet. Per unit of feed, the cost of protein in the diet is quite high and therefore reducing the CP level may reduce the overall cost of the diet. However, the long term effects of lowering diet CP level to 14.9% on milk production and the possible influence on reproductive performance are unknown. Full lactation trials are needed to confirm wide spread use of a low protein diet and to investigate the use of such diets in early lactation cows.

6. GENERAL DISCUSSION

The efficiency with which ruminants utilize dietary N leads to the excretion of excess dietary N in feaces and urine (Tamminga 1992; Gaylean 1996; Bierman 1999). This loss of dietary N is expensive in terms of both environmental pollution (Socolow 1999; Janzen et al. 2003; Hristov et al. 2011) and its impact on the cost of ruminant production (Chandler 1996). Therefore, improving the efficiency of N utilization in ruminants not only benefits producers with potential cost savings but also offers the opportunity to reduce the environmental impact of intensive livestock operations through incorporating sustainable agricultural practices.

The recycling of urea-N to the GIT in ruminants is a salvage mechanism that conserves N during low dietary N conditions (Lapierre and Lobley 2001). Without this mechanism N would be irreversibly lost to the animal and could result in a N deficiency (Stewart and Smith 2005). By increasing urea-N recycling to the GIT there would be more N available for ruminal microbial protein production which could be utilized for anabolic purposes by the animal (i.e., meat and milk production) (Lapierre et al. 2006), improving N efficiency. Both dietary and ruminal factors have been shown to affect the rate at which urea-N is recycled to the GIT (Kennedy and Milligan 1980; Huntington et al. 1989; Marini and Van Amburgh 2003; Abdoun et al. 2010). There is limited information on how concomitant changes in certain dietary factors affect the recycling of urea-N to the GIT and its subsequent incorporation into microbial protein. Therefore, the goal of this thesis was to investigate how manipulation of dietary CP, RDP, and RFC levels can influence urea-N recycling in ruminants with the aim of improving overall N efficiency. Three experiments were conducted. The first investigated the interactive effects of dietary CP level and degradability on urea recycling, microbial protein production, and nitrogen balance (Chapter 3); the second investigated the effects of dietary RDS and RDP levels on urea recycling, microbial

protein production, nitrogen balance, and duodenal nutrient flow (Chapter 4). Both used beef heifers as experimental models. The final experiment investigated the effects of dietary CP and RDP levels in lactating Holstein dairy cows on urea recycling, microbial protein production, nitrogen balance, omasal nutrient flow, and milk production (Chapter 5).

Reducing dietary N level has been shown to increase urea-N recycling to the GIT and reduce overall N excretion to the environment (Marini and Van Amburgh 2003). Furthermore, dietary RDP level affects ruminal NH_3-N concentration (Wickersham et al. 2008a) which, in turn, influences urea-N transfer to the GIT (Kennedy and Milligan 1980). In Chapter 3, reducing dietary CP level from 14.0 to 10.8% (on DM basis) reduced both ruminal NH_3-N concentration and endogenous production of urea-N. Substantial amounts of urea-N were recycled to the GIT but the absolute amounts remained similar across diets with most urea-N subsequently being returned to the ornithine cycle. It has previously been demonstrated that decreasing ruminal NH_3-N concentration increases urea-N transfer to the GIT (Marini ad Van Amburgh 2003; Marini et al. 2004). This indicates that in beef heifers (Chapter 3) urea-N escaped incorporation into microbial protein and therefore was not available to the animal. This offers the opportunity to further enhance the efficiency of N utilization of ruminants fed low CP diets by directing more urea-N to anabolic purposes.

In Chapter 3, lowering dietary CP level to 10.8% (on DM basis) did not negatively impact diet digestibility or microbial protein production. Microbial protein is an important source of AA and can provide 60% to 80% of metabolizable protein (NRC 2001) which can be used for productive purposes. This finding is important and suggests that microbial protein sythesis can be maintained at a dietary CP level of 10.8%. If microbial protein production can be sustained when feeding rations formulated to 10.8% CP and translated into sustained animal growth and

production, substantial advances could be made in reducing N excretion into the environment as well reducing dietary protein costs. However, animal growth trials would need to be conducted to investigate whether or not the maintenance of microbial protein production on low CP diets translates into enhanced growth and production in beef cattle.

Furthermore, feeding a reduced dietary CP level was associated with a 30% decrease in urinary N excretion. The excretion of excess dietary N, particularly as urinary urea-N (Reynal and Broderick 2005) can cause environmental degradation as urea-N is rapidly volatilized to ammonia, which can subsequently be converted to NO_2 and N_2O causing environmental pollution (Hristov et al. 2011). However, GER to faeces was significantly greater in heifers fed the low CP diet compared with high CP diet. Since, faecal N is less volatile than urinary N, there is opportunity to reduce environmental pollution by partitioning more excreted N to faeces rather than urine (de Klein and Ledgard 2005). If this management strategy of reducing dietary CP level was incorporated into intensive livestock operations, particularly those feeding a large number of animals, it could result in a significant decline in N excretion and reduce the potential negative environmental impact of such production systems.

Ruminal microbes require ruminally-fermentable carbohydrate in order to incorporate ruminal NH_3-N into microbial protein (Nocek and Russell 1988). Therefore altering the supply of both ruminally-fermentable carbohydrate and RDP may affect microbial incorporation of urea-N returned to the GIT and increase microbial protein reaching the small intestine. In Chapter 4, total N flow to the duodenum was unaffected by dietary treatment however, when feeding the high RDS, increasing RDP level increased both microbial N flow to the duodenum and microbial efficieny in accordance with previously published data (Herrera-Saldana et al. 1990; Aldrich et al. 1993; Cruz Soto et al. 1994). These results can be attributed to the judicious

combination of dietary ingredients resulting in the synchrony of energy and NH_3-N release in the rumen. Microbial protein closely matches the amino acid profile of beef and milk (Storm and Ørskov 1983). Therefore optimizing ruminal microbial production is important in terms of supplying amino acids to the host animal.

Resluts from Chapter 4 showed that by reducing dietary N level and through feeding a judicious balance of RDS and RDP, overall N efficiency in beef heifers can be improved, whilst still maintaining microbial protein supply to the animal. Although further investigation is required, the incorporation of such feeding strategies by producers could result in lowered feed costs and a reduction in the negative environmental impact from the excretion of excess dietary N whilst still maintaining productive performance. These are important factors to consider when deciding to incorporate new feed formulation approaches on farm. Furthermore, agricultural practices that enhance the stewardship of the environment and promote sustainability are crucial to abating public concerns over the impact of intensive livestock production systems (Steinfeld et al. 2006; de Klein et al. 2010; Janzen 2011).

Lactating dairy cows are normally fed relatively high CP diets (17.5 to 19.0% on DM basis; NRC 2001). As with beef heifers, the overfeeding of dietary CP to lactating dairy cows results in the excretion of excess dietary N into the environment potentially causing pollution. However, in dairy cows increasing dietary CP level also leads to an increase in MUN level (Canfield et al. 1990). Milk urea N levels exceeding 19 mg dL^{-1} are linked to a reduction in pregnancy rate (Butler et al. 1993) as well as reduced fertility (Larson et al. 1997) in lactating dairy cows. Feeding strategies that reduce dietary CP intake whilst still maintaining productive performance are essential to improving the efficiency of N utilization in lactating dairy cows. In Chapter 5, results showed that reducing dietary CP level from 17.5 to 14.9% not only

significantly reduced urinary excretion of N but also improved milk N efficiency, as there was no effect of treatment on milk yield. This provides the opportunity to reduce the impact of excess N excreted into the environment whilst at the same time reducing overall feed costs. The data suggested that milk yield may not have been affected by treatment due the maintenance of microbial protein production, even on the low CP diet. Whilst these results are promising it is unknown whether or not lactating dairy cows would be able to maintain milk yield throughout lactation or during early lactation when fed diets containing 14.9% CP. It is also of significance to note that lowering dietary CP level reduced MUN level, a factor shown to influence pregnancy rate and fertility in lactating dairy cows. Reduced fertility of dairy cows can be costly to producers, therefore, investigating the impact of low CP diets on reproductive factors, such as conception and pregnancy rates may further enhance the adoption of such feeding strategies within the dairy industry.

7. OVERALL CONCLUSIONS

This thesis provides data relating to improving the efficiency of N utilization through enhanced urea-N recycling in ruminants. The results presented here show that reducing dietary CP levels, for both beef heifers and dairy cows reduces the excretion of urinary N. Furthermore, on low CP diets, microbial protein production was, at the very least, maintained and this was partially mediated by effects on urea-N recycling to the GIT due to the judicious combinations of dietary RDS and RDP. The data presented in this thesis is from short-term trials. Additional information is required to elucidate the impact of these dietary manipulations over an entire production cycle.

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